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BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

XII. SOILS AND VEGETATION OF TWO CULTIVATED SITES

By R. H. THORNTON, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(Received for publication, 24 November 1959)

Summary

As a background to subsequent biological investigations a description is given of the soils and natural vegetation at two sites under low-tussock grassland. Details of introduced pasture and crops established on this tussock grassland are summarised for comparison.

INTRODUCTION

A series of investigations is being undertaken in these laboratories to gain a broad knowledge of the micro-organisms present in New Zealand soils and their ecological relationships. These investigations are regarded more as initial or reconnaissance surveys than final or complete accounts. It is hoped such studies will provide a background of knowledge to which more detailed studies can later be added.

Several groups of micro-organisms occurring in three tussock-grassland soils in New Zealand have already been examined (Thornton 1958a, b; di Menna 1958; Stout 1958a, b; Ross 1958a, b; Vernon 1958; Flint 1958). The present investigation was initiated to determine to what extent some of these micro-organisms are influenced by the cultivation and agricultural development of tussock-grassland soils. Reactions to such changes of the soil environment might contribute to knowledge of the ecology of soil micro-organisms. Parallel observations have been made of the fungi, yeasts, bacteria, and protozoa occurring in uncultivated and cultivated tussock-grassland soils. An account of the sample sites is given below. Results of studies on particular groups of micro-organism will be given in subsequent parts of this series.

SITES AND SOILS

Investigations were carried out at two localities, one in central North Island near Waiouru (map ref., 1-mile sheet, N122.157397,

N132.175377), on Taupo hill soil, on a farm 3–4 miles south from the location of an earlier similar biological investigation of tussock-grassland soils (Thornton 1958a), and the other in South Island, near Broken River (map ref., 4-mile sheet, N.Z.M.S. 10B, Sheet 25, grid square 55, 2098) on Cass fine sandy loam, 10 miles distant from the location of an earlier similar biologial investigation of tussock-grassland soils on Black Range, near Bealey (Thornton 1958a).

A description of the Taupo hill soil near Waiouru is as follows:

6 in. very dark grey sandy silt; friable; weakly developed fine granular structure; many roots; very few pumice stones; diffuse boundary;

6 in. dark brown fine sandy loam; friable; weakly to moderately developed nutty structure; gradual boundary;

on dark yellowish brown fine sandy loam with few pumice stones.

An account of the relationship of this soil to the genetic classification of New Zealand soils has already been given by Thornton (1958a), namely a yellow-brown pumice soil; it is very weakly weathered.

The site near Broken River was on the Broken River experimental plots of the N.Z. Department of Agriculture, situated on an old river terrace at an altitude of 2,350 ft in the Castle Hill basin. A description of the soil, Cass fine sandy loam, is as follows:

4–5 in. dark brown fine sandy loam; friable; weakly developed medium and fine nutty and fine crumb structure;

12 in. yellowish brown sandy loam, friable; weakly developed fine block structure;

on pale yellowish grey silt.

This soil is included in the yellow-brown earth group of Taylor (1948), and was originally formed under low-tussock grassland on greywacke alluvium. It is weakly to moderately weathered and moderately to strongly leached.

Vegetation

The vegetation of the uncultivated tussock-grassland sites at Waiouru was similar to that already described for another site at Waiouru (Thornton 1958a), namely, *Festuca novae-zelandiae* physiognomically dominant, with other grasses, flatweeds, and herbs, forming a dense inter-tussock layer. The vegetation of the cultivated sites is indicated below. The vegetation of the uncultivated tussock-grassland site at Broken River was a low-tussock grassland broadly similar to that at Bealey. An account of the vegetation of the Castle Hill basin, which includes the Broken River area, was given by Relph (1957). *Festuca novae-zelandiae,* a small (1–2 ft high) tussock grass, is physiognomically dominant although individual plants may be up to 1–2 ft apart. Sweet vernal (*Anthoxanthum odoratum*) and browntop (*Agrostis tenuis*) are dominant in the inter-tussock vegetation, which also includes small native herbs and clovers. The plant cover on the cultivated sites is indicated below.

SAMPLE SITES

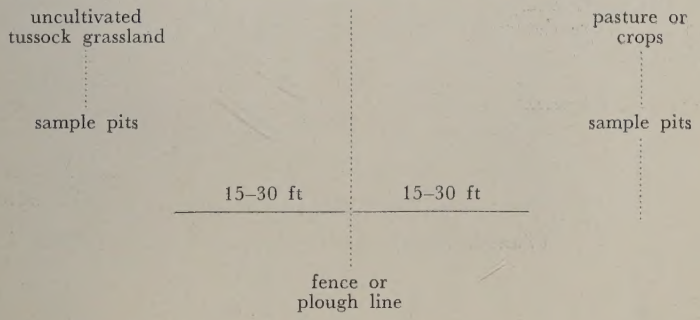
Investigations were made in tussock-grassland soils managed at three stages of agricultural use:

(a) uncultivated tussock grassland subject to light grazing by sheep —tussock site.

(b) cultivated former tussock grassland, ploughed, sown with crops and grazed by sheep—crops site.

(c) cultivated former tussock grassland, sown with introduced grasses after cultivation, and grazed by sheep—pasture site.

Investigations were planned as a series of comparisons between uncultivated and cultivated soils, sample pits, at 15–30 ft intervals, being paired along the boundary between sites as shown below:



The agricultural history of the sites is given below:

Waiouru (Taupo hill soil)

Crops Site (1 year old). Ploughed in 1954, sown with swedes and grazed. Sown with soft turnips in 1955 and grazed. Superphosphate applied annually at a rate of 1–2 cwt per acre.

Pasture Site (18 years old). Ploughed and sown with introduced grasses about 1937. Present pasture dominated by sweet vernal and crested dogstail (*Cynosurus cristatus*) with Yorkshire fog (*Holcus lanatus*) and white clover (*Trifolium repens*) subdominant. No phosphate fertiliser applied since 1937. Little is known of the previous history.

Broken River (Cass fine sandy loam)

Crops Site (1 year old). Ploughed in April 1954 and sown in October 1954 with swedes, turnips, kale and chou moellier. Fertilised with 15 cwt lime, 3 cwt of a mixture (3 : 1) of reverted superphosphate (reverted with serpentine) and nitrolime, and 10 lb borax per acre.

Pasture Site (1 year old). Ploughed in April 1954 and sown in October 1954 with introduced grasses and clovers (perennial ryegrass 15 lb, short-rotation ryegrass 5 lb, cocksfoot 5 lb, tall oat grass 5 lb, timothy 3 lb, Montgomery red clover 1 lb, alsike clover 1 lb, white clover 2 lb, and sheep's burnett 3 lb per acre. Fer-

tilisers were applied in 1954 (15 cwt lime, 2 cwt superphosphate, 1 cwt nitrolime, 2½ oz sodium molybdate per acre) and in 1955 (2 cwt superphosphate, 15 cwt lime per acre). An application of DDT, at an unknown rate, was made in 1955.

In the crops sites at both locations the remains of the old tussock plants were plentiful with still quite large lumps of the old sod buried in the soil.

A general description of agricultural practices at Waiouru was given by Moss (1956) and an account of the work at the Broken River experimental plots was given by Dingwall (1956).

METHODS

Chemical analyses and temperature and moisture relations of the two soils were determined as before (Thornton 1958a), and are given in Table 1.

TABLE 1. CHEMICAL ANALYSES AND TEMPERATURE AND MOISTURE RELATIONS OF UNCULTIVATED AND CULTIVATED TUSSOCK-GRASSLAND SOILS

	Taupo Soil (Waiouru)				Cass Soil (Broken River)		
	Tussock	Pasture	Tussock	Crops	Tussock	Pasture	Crops
pH (glass electrode)	5.8	5.6	5.8	5.3	5.1	5.2	5.2
Organic carbon %	5.6	7.2	5.0	5.9	3.1	3.7	3.0
Total nitrogen %	0.45	0.69	0.44	0.47	0.23	0.27	0.23
C/N ratio	12	10	11	13	13	14	13
Citric-acid-sol P. mg % P	13	23	16	18	11	14	13
Exch. capacity. me. %	20.7				19.8		
Total bases me. %	10.3				7.0		
Exch. Ca me. %	6.6				5.1		
*Soil temp. °c spring	11.7	11.8	11.7	11.5	14.6	15.0	15.8
summer	20.7	22.0			16.2	20.0	
†Soil moisture % d.w. spring	47.7	51.5	53.4	53.2	37.3	34.1	30.8
summer	38.8	32.2			10.2	16.4	

* Means of several readings taken at the beginning and the end of each sampling period at the 3-in. level.

† Means of triplicate samples collected at the 2–4 in. level at the beginning and end of each sampling period.

Analyses by M. Fitzpatrick and L. L. Horne according to the methods of Metson (1956).

The methods of study were similar to those already described (Thornton 1958). Five sample pits were dug at each site. Samples were taken and observations were made in spring, 19-25 October 1955, and in summer, 24-28 February 1956, at both locations. At the tussock sites inter-tussock zone samples only were taken at a depth of 2–4 in. At crops and pasture sites samples were taken from the 2–4 in. layer. (The sites under crops were not available for investigation in the summer

owing to normal crop rotation practices.) At Waiouru comparisons between sites under tussock and crops and between tussock and pasture were made at two separate locations, half a mile apart.

Some of the chemical changes in the soil that followed agricultural development of these tussock areas can be seen in Table 1. In the Taupo soil at Waiouru, under the 18-year-old pasture, there was a slight increase in pH; phosphorus soluble in 1% citric acid increased markedly, probably from organic phosphorus held up in the organic matter, and carbon and nitrogen contents increased very markedly. The greater relative increase of nitrogen caused a lessening of the C/N ratio. This increase in nitrogen probably results from the introduction and establishment of white clover in an area naturally low in leguminous plants. These figures confirm previous findings on a Taupo pumice soil (Walker 1956). Taupo soil under crops at Waiouru, and Cass soil under crops and under pasture at Broken River, showed little chemical change from the tussock soil. Possibly the changes were too recent to affect the chemical properties in these sites. In general it can be said that these soils have a low level of fertility.

ACKNOWLEDGMENTS

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BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

XIII. FUNGI OF TWO CULTIVATED SOILS

By R. H. Thornton, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 24 November 1959*)

Summary

The fungi occurring in two cultivated tussock-grassland soils were examined, employing screened-immersion plates. Fungal populations were little altered qualitatively when the tussock vegetation was replaced by crops or by introduced pasture. Numbers of mycelia isolated increased under crops.

Introduction

In an earlier investigation (Thornton 1958b), the fungal flora of three naturally occurring tussock-grassland soils was described and may be regarded as the autochthonous or true soil fungal population. The present study was a preliminary investigation made to determine to what extent this soil fungal population was influenced by agricultural development involving cultivation and alteration of higher-plant cover. Indications were sought of any major influences of these cultural changes on the autochthonous flora of tussock-grassland soils. The investigation did not include a detailed study of the immediate effects of these changes on the soil flora or of the succession of fungi occurring at intervals after the changes.

Sites, Soils and Vegetation

A description of the soils and vegetation at two sites selected for study is given by Thornton (1960).

Methods

The fungal populations occurring in two cultivated tussock-grassland soils were examined by the screened-immersion plate method (Thornton 1952) with modifications (Thornton 1956). At each site 10 screened plates, 2 in each of 5 pits, were buried at a depth of 2–4 in. for 7 days in spring and 4 days in summer. Only 5 plates were used in Taupo soil in spring in the tussock-pasture sites. In the tussock sites the soil between spaced tussock plants only was sampled—the inter-tussock zone (Thornton 1958a). Crop sites were sampled in spring only while the pasture sites were sampled in both spring and summer.

Results and Discussion

Although a number of fungi were isolated from both soils, only the more commonly occurring species have been considered. The occurrence of fungi frequently isolated from the two soils examined is given in Table 1, while a list of the species isolated is given in the Appendix.

TABLE 1. OCCURRENCE OF FUNGI FREQUENTLY ISOLATED BY SCREENED-IMMERSION PLATES FROM UNCULTIVATED AND CULTIVATED TUSSOCK-GRASSLAND SOILS

Fungus	Taupo Soil				Cass Soil			
	Spring		Spring and Summer		Spring		Spring and Summer	
	Tussock	Crop	Tussock	Pasture	Tussock	Crop	Tussock	Pasture
Rhizoctonia sp.	7	14	31	36	19	61	30	37
Fungus S.B. 235	1	—	23	32	5	—	5	1
Papulaspora sp.	13	2	19	—	6	2	7	4
Other non-sporing mycelia	2	2	2	4	3	8	6	13
Zygorhynchus moelleri	1	3	3	3	4	5	36	28
Cylindrocarpon radicicola	8	17	14	21	1	2	2	2
Fusarium sambucinum var. *coeruleum*	—	8	6	9	—	3	2	9
Trichoderma viride	—	1	4	7	2	—	3	8
Mucor hiemalis	—	4	3	3	2	2	2	5
Penicillium janthinellum	—	—	2	—	1	1	5	16
Mortierella alpina	—	—	1	4	—	1	10	7
Mortierella elongata	—	1	—	—	2	1	11	3
Penicillium stoloniferum	—	—	—	—	4	—	4	1
Penicillium frequentans	—	—	—	—	1	4	1	2
(See Appendix for other fungi isolated)								
Total numbers of mycelia isolated	35	55	117	126	60	93	141	148
Mean number of mycelia isolated per screened-immersion plate	3.5	5.5	7.8	8.4	6.0	9.3	7.1	7.4

Results from observations in spring and summer on the tussock-pasture sites in both soils have been grouped together.

The fungal populations found in the tussock soils in the present investigation were very similar to the fungal population found in tussock soils nearby in an earlier, similar investigation (Thornton 1958b); non-sporing mycelia were predominant, particularly *Rhizoctonia* sp., together with *Papulaspora* sp., Fungus S.B. 235, and other non-sporing mycelia: *Cylindrocarpon radicicola* and *Trichoderma viride* were also frequently isolated from the cooler South Island Cass soil.

The fungal population of soils formerly covered by tussock grassland and now cultivated and sown with crops or pastures, showed few changes compared with the population from tussock soils. In both Taupo and Cass soils more mycelia were isolated from under crops than from under tussock. Employing the dilution plate method, Brierley (1923) found the numbers of fungi higher in cultivated than

in virgin soils while Guillemat and Montegut (1956) found that ploughing caused an increase in fungal counts. The increase in numbers of mycelia in the crops sites may have been due to the increased soil aeration resulting from cultivation, from the change in the type and density of vegetative cover, or from the greater additions of dung and urine as a result of the greater number of sheep grazed on these sites. In both Taupo and Cass soils mycelia of *Rhizoctonia* sp. were isolated more frequently from under crops and so were mycelia of *Fusarium sambucinum* var. *coeruleum*. Mycelia of *Cylindrocarpon radicicola* were more common under crops in Taupo soil. *Papulaspora* sp. was relatively less frequently isolated from soil under crops than from under tussock, particularly in Taupo soil.

In both Taupo and Cass soils, isolations of mycelia from former tussock soils now under a pasture cover were very similar qualitatively to isolations from tussock soils. Increased isolations of mycelia were recorded under pasture of *Cylindrocarpon radicicola* in Taupo soil and of *Penicillium janthinellum* and other non-sporing mycelia in Cass soil. *Papulaspora* sp. showed a marked decline in occurrence under the 18-year-old pasture on Taupo soil. Under the younger pasture on Cass soil the decline in occurrence was not very strong. *Mortierella elongata* appeared to be less frequent under pasture in the Cass soil.

Although some of the changes recorded above were quite marked, the significance of the smaller changes in the numbers of isolations of mycelia of other species is not known. It would appear that in both soils, apart from the decrease in numbers of isolations of mycelia of *Papulaspora* sp. and the tendency of isolations of *Fusarium sambucinum* var. *coeruleum* to increase, there were no marked changes following the agricultural development of these two former tussock-grassland soils. This suggests that the changes in vegetative cover, which, under the 18-year-old pasture on Taupo soil, resulted in changes in phosphorus content, C/N ratio, etc. (Thornton 1960), may not have involved sufficiently profound alterations in the type of organic matter available to micro-organisms for a marked change to occur. The organic additions from introduced pasture plants, which will largely be of root origin, probably did not differ very widely from the organic additions from root material from a tussock-grassland cover. The effects of organic additions from crops were probably masked by the plentiful remains of old tussock plants in these cultivated soils.

It is not known if there was any change in the physiological activities of the more common fungal species following the changes in vegetative cover.

The increase in organic matter content of the Taupo soil under 18-year-old pasture would be expected to cause an increase in microbiological activities which would be reflected in increased numbers of micro-organisms. The fact that the numbers of mycelia isolated from under pasture were only a little higher than mycelial isolations under tussock suggests that much of the organic matter was unavailable to fungi. This may be associated with the high absorption of organic matter in this soil thought to be caused by the high proportion of allophane in the clay fraction (Fieldes 1955).

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List of Fungi Isolated from Uncultivated and Cultivated Tussock-grassland Soils by Screened-immersion Plates

Phycomycetes

Mucor hiemalis Wehmer
Zygorhynchus moelleri Vuill.
Mortierella alpina Peyronel.
M. elongata Linm.
M. horticola Linm.
M. minutissima V. Tiegh.
M. polycephala Coem.
M. spinosa Linm.
M. traversiana Peyr.

Ascomycetes

Arachniotus sp.

Yeasts

Candida humicola (Daszewska) Diddens et Loder

Fungi Imperfecti

Streptomyces sp.
Trichoderma viride Pers. ex Fr.
Penicillium citrinum Thom
P. cyclopium Westl.
P. frequentans Westl.
P. janthinellum Biourge
P. nigricans (Bain.) Thom
P. soppii Zaleski
P. stoloniferum Thom
P. thomii Maire
Paecilomyces sp.
Gliocladium roseum (Link.) Thom
Verticillium sp.
Humicola grisea Traaen
Hormodendron cladosporoides (Fr.) Sacc.
? *Torula* sp.
Trichocladium opacum (Corda) Hughes
Stemphylium sp.
Alternaria sp.
Fusarium sambucinum Fuckel var. *coeruleum* Wr.
F. oxysporum Schlecht emend. Snyder et Hansen.
Cylindrocarpon radicicola Wr.
Rhizoctonia sp.
Papulaspora sp.
Fungus S.B. 235
Other non-sporing mycelia

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

XIV. YEASTS OF TWO CULTIVATED SOILS

By M. E. di Menna, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 24 November 1959*)

Summary

Yeasts in two tussock-grassland soils, under native tussock, under introduced pasture, and under crops, were examined quantitatively and qualitatively. In the first soil the only change that occurred when the tussock was brought into cultivation was a slight increase in yeast numbers. In the second, the yeasts in the cultivated sites showed a diminution in numbers and some change in species pattern. In both soils the yeast counts were higher in summer than in spring. There was evidence that *Candida humicola* was associated with the roots of tussocks but not with the roots of introduced pasture plants. *Cryptococcus laurentii* was the commonest yeast in washings from the leaves of both tussock and pasture plants collected in the spring.

Introduction

A qualitative study of yeasts in uncultivated tussock-grassland soils (di Menna 1958) showed that only a few species could be isolated from this habitat, that these species were common to the soils of the three widely separated localities studied, and that a different species was dominant in each locality. The following is an account of a quantitative and qualitative study of the yeast flora of two tussock-grassland soils that had been brought under cultivation. One of these, Taupo hill soil at Waiouru, had been sampled in the previous survey. In the present work it was sampled at four sites. One had been under introduced pasture plants (sweet vernal, crested dogstail, Yorkshire fog, white clover) for eighteen years. The second had been sown with crops for the last two years. Two sites under tussock were sampled, one close to the site under introduced pasture and one close to the site under crops. The tussock sites had never been cultivated but were invaded by introduced pasture plants.

The second soil, Cass fine sandy loam at Broken River, was sampled at three sites, one under tussock, one under introduced pasture and one under crops. The pasture and crops had been established the previous year.

Materials

A full description of the soils, sites, and methods of sampling is given by Thornton (1960a).

Five soil samples were taken at each of the following sites. Taupo soil, spring 1955: site under pasture, site under crops, two parallel sites under tussock. Taupo soil, summer 1955-56: site under pasture, parallel site under tussock. Cass soil, spring 1955: site under pasture, site under

crops, site under tussock. Cass soil, summer 1955-56: site under pasture, site under tussock.

Samples of tussock leaves and leaves of pasture plants were taken from Waiouru in the spring of 1956. Turfs of tussocks and of pasture plants were collected from Waiouru at the same time so that roots could be dissected out in the laboratory.

Methods

Glucose peptone agar brought to a pH of 4, as used in the first part of this work (di Menna 1958), was the medium on which primary isolations were made. The poured plates were dried out for a short time at *ca.* 70°c in order that the medium should absorb the water in the inoculum.

Each soil sample was cultured separately. Ten grammes were taken from each sample with sterile precautions and made into serial dilutions of 1/25, 1/250, 1/500, and 1/1,000. The inoculum for each plate was 0.5 ml which was spread over the surface of the dried medium. Three plates were inoculated from each 1/250 dilution, three from each 1/500 and four from each 1/1,000. Cultures were incubated at room temperature for 4–6 days before counts were made. Twenty colonies per soil sample were subcultured from the Taupo soil spring collection and the Cass soil summer collection. Ten colonies per sample were taken from the other soil collections.

The pasture plant and tussock leaves from Waiouru were shaken with sterile tapwater and the washings spread on pH 4 glucose peptone agar plates. Twenty yeasts were subcultured from each of the two sets of cultures. The pasture plant and tussock roots were dissected out from the turfs with sterile forceps and treated in the same manner as the leaves.

Cultures were identified by the methods and criteria of Lodder and Kreger-van Rij (1952). *Pullularia pullulans* (De Bary et Low) Berkhout was treated as a yeast.

The majority of the isolates were easily identifiable. Isolates of a given species from the same site were usually very similar and did not exhibit the variation often found amongst isolates of the same species recovered from a substrate in which they occur casually as contaminants. One isolate could not be identified and was labelled unknown. Two strains of *Cryptococcus laurentii* (Kufferath) Skinner recovered from Cass soil under pasture in the spring caused some difficulty. When first isolated they grew so feebly that it was impossible to identify them by standard procedures. They were subcultured on liver extract agar and potato glucose agar but at first showed no visible growth. However, after three months, small secondary colonies appeared on all subcultures of both strains. These, when transferred to fresh media, grew normally and were identified. Possibly the original isolates were naturally occurring, unstable, lethal variants (Mackinnon 1940). A similar phenomenon was seen in a strain of *Candida albicans* (Robin) Berkhout (di Menna 1953).

Results

Results are given in full in Tables 1, 2, and 3.

Yeast counts from the Taupo soil under cultivation were somewhat higher than from the soil still under tussock, both in spring and in summer. The average spring count from the two tussock sites was 6,500 yeasts/g (wet weight) of soil, from the pasture site, 8,600, and from the site under crops, 12,200. Counts in individual soil samples varied between 500 and 22,000/g, both of these extremes being in the tussock site parallel with the crops. In the summer the average count from the pasture was 23,000 and from the tussock site parallel with it, 21,000.

TABLE 1. Yeasts Isolated from Taupo Soil at Waiouru
(Percentages of different species)

	Spring				Summer	
	Tu*	Pa	Tu	Cr	Tu	Pa
Debaryomyces kloeckeri Guill. et Peju	—	—	—	—	2	—
Cryptococcus laurentii	—	1	—	2	8	4
Cryptococcus albidus	40	25	22	19	18	18
Cryptococcus diffluens	3	1	1	2	12	6
Cryptococcus terreus	49	58	66	66	56	66
Torulopsis sp.	—	1	—	—	—	—
Candida parapsilosis (Ashf.) Langeron et Talice	2		—	—	—	
Candida curvata	1	—	3	6	4	—
Trichosporon cutaneum	5	14	4	5	—	6
Rhodotorula glutinis (Fres.) Harrison	—	—	2	—	—	—
Rhodotorula mucilaginosa (Jorg.) Harrison	—	—	2	—	—	—
Total yeasts isolated	100	100	93	100	50	50
Average count/g in thousands	6.8	8.6	6.3	12.2	21.0	23.0
Range of count/g in thousands	4–11	4–16	0.5–22	5–18	4–35	6–38

* Tu, tussock; Pa, pasture; Cr, crops.

The species pattern in Taupo soil at all four sites was very similar. Only three species occurred in important numbers, *Cryptococcus terreus* di Menna (60% of total isolates), *Cryptococcus albidus* (Saito) Skinner (25%), and *Trichosporon cutaneum* (de Beurm., Gougerot et Vaucher) Ota (6%).

In Cass soil the average yeast counts were lower than in Taupo soil, both in spring and summer. Total numbers of yeasts did not increase with cultivation; average counts from the sites under introduced pasture and under crops were lower than those from the site under tussock. *C. albidus* was the dominant species in the soil under tussock

TABLE 2. YEASTS ISOLATED FROM CASS SOIL AT BROKEN RIVER
(Percentages of different species)

	Spring			Summer	
	Tu*	Pa	Cr	Tu	Pa
Sporobolomyces roseus Kluyver et van Niel	—	—	—	—	1
Cryptococcus laurentii	—	30	4	—	29
Cryptococcus albidus	46	34	34	44	24
Cryptococcus luteolus (Saito) Skinner	—	—	6	—	—
Cryptococcus diffluens	—	4	2	2	4
Cryptococcus terreus	34	18	32	44	28
Torulopsis ingeniosa di Menna	—	4	—	—	—
Candida scottii Diddens et Lodder	—	—	—	—	1
Candida curvata	—	8	8	8	1
Trichosporon pullulans (Lindner) Diddens et Lodder	—	—	—	—	2
Trichosporon cutaneum	18	—	4	2	5
Rhodotorula rubra (Demme) Harrison	—	—	4	—	1
Rhodotorula mucilaginosa	—	2	6	—	2
Pullularia pullulans	2	—	—	—	1
Unknown	—	—	—	—	1
Total Yeasts Isolated	50	50	50	100	87
Average count/g in thousands	4.6	4.6	3.8	5.7	5.0
Range of count/g in thousands	2–9	2–6	1–6	3–12	1–10

* Tu, tussock; Pa, pasture; Cr, crops.

(45% of the isolates), and *C. terreus* the next most frequently occurring (39%). Only four other yeast species were isolated from this site. A total of fifteen species were isolated from the sites under crops and under pasture. Most of these occurred in small numbers only, but in the site under pasture *C. laurentii* was found in proportions as great as or greater than *C. albidus* and *C. terreus*.

C. laurentii was the dominant yeast on the leaves of both tussock and pasture plants at Waiouru. *Candida humicola* (Daszewska) Diddens et Lodder made up ten of the twenty yeast isolates from the root washings of tussocks at Waiouru, but was not recovered from the pasture-plant root washings.

DISCUSSION

Numbers of yeasts in these soils are of the same order as those reported elsewhere. Starkey and Henrici (1927) in the United States found very few yeasts in the soils they examined, but unpublished observations by Miller, Peers, and Neal, quoted by Miller and Webb (1954), indicated that there were about 5,000 yeasts/g in Canadian

TABLE 3. Yeasts Isolated from Roots and Leaves of Tussocks and Pasture Plants at Waiouru

	Roots		Leaves	
	Tu	Pa	Tu	Pa
Cryptococcus laurentii	—	1	18	14
Cryptococcus albidus	1	3	—	—
Cryptoccus terreus	4	12	—	—
Torulopsis ingeniosa	1	—	—	4
Candida humicola	10	—	—	—
Trichosporon cutaneum	4	4	—	—
Rhodotorula rubra	—	—	-	1
Rhodotorula mucilaginosa	—	—	1	—
Rhodotorula marina Phaff, Mrak, et Williams	—	—	—	1
Pullularia pullulans	—	—	1	—
Total yeasts isolated	20	20	20	20

meadow soil and 4,000/g in forest soil. In samples of other Canadian soils Miller and Webb found an average of 1,000 yeasts/g and a maximum of approximately 50,000. Lund (1954) found varying numbers in Danish soils, from none in seashore samples to 188,000 in surface samples from an orchard. The counts in the tussock grassland may be among the lower range for New Zealand soils, for from 6,000 to 244,000 yeasts/g were isolated from a yellow-brown earth under introduced pasture in the Wellington district (di Menna 1957).

Although there were variations in the numbers of yeasts from sample to sample at each site, the trend of the counts was consistent. For all sites at both localities the averages of the summer counts were higher than the averages for the spring counts. Of all sites sampled more than once, the Taupo soil under pasture had the highest average count for the spring and for the summer, and the Cass soil under pasture the lowest. This consistency of trend was not found in the yellow-brown earth already mentioned, in which there were large quantitative and qualitative fluctuations from sample to sample and in which no seasonal change in numbers could be seen. It was thought that the sample variation here was due to the discontinuous effect of rotted forest remains in an unploughed soil, and that either no seasonal fluctuation in yeast numbers occurred in an area where there were only small variations in moisture and temperature throughout the year, or else that the seasonal change was obscured by sample variation. An even cover of tussock and a wider range of temperature with season are probably the reasons for the small sample variation and the seasonal change in numbers in the Taupo and Cass soils.

The pattern of yeasts in the Taupo soil sites sampled in this survey was like that of the Taupo soil site examined previously (di Menna 1958). *C. terreus* was dominant and *C. albidus* occurred in important numbers. This suggests that the yeast flora does not vary within a soil type.

The species dominant in the Cass soil under undisturbed tussock, *C. albidus* and to a less extent *C. terreus,* were not quite the same as those at a previously sampled tussock site of Tekoa soil, ten miles away (di Menna 1958). Here *Candida curvata* (Diddens et Lodder) Lodder et van Rij was dominant and *Cryptococcus albidus* subdominant. Cass and Tekoa soils are closely related and the divergence in yeast floras may be due to the different vegetational histories of the two localities. Beech forest covered the Tekoa soil site until recent times and, as far as is known, has never been present on the Cass soil site. *Candida curvata* is a species which seems to be typical of soil under forest, and the nitrate-assimilating *Cryptococcus* spp., *C. albidus, C. terreus,* and *Cryptococcus diffluens* (Zach) Lodder et van Rij, appear to be typical of grassland soils. In the Tekoa soil it seems likely that the original forest dominant, *Candida curvata,* is being replaced by *Cryptococcus albidus,* the species characteristic of the soils of the area which have been always under tussock.

Effects of Cultivation

The results seem to give conflicting evidence about the effects of cultivation on the soil yeast flora. The data from the Taupo soil suggest that, apart from a small but consistent increase in numbers, cultivation does not affect the soil yeast flora, either initially when the tussock is first ploughed under and planted with crops, or after a number of years when an introduced pasture is fully established. Contrary to this, in the Cass soil, yeast numbers did not change, or decreased slightly in the cultivated sites, and not only was there an increase in numbers of species isolated but also *C. laurentii,* a species not found in the soil under tussock, became numerically important. It appeared as if conditions had become unfavourable for the dominant yeasts and that they had been partially replaced by species usually restricted to the surfaces of grass leaves. A somewhat similar effect was seen in the bacterial population (Stout 1960a), but not in the moulds of protozoa (Thornton 1960b; Stout 1960b).

Yeasts Associated with Grasses

The dominance of *C. laurentii* on the leaves of both tussocks and introduced pasture plants at Waiouru suggested the source of this yeast in the samples of Cass soil under cultivation. A detailed study has shown that yeasts on leaves of pasture plants vary largely with season but not with locality (di Menna 1959).

There is further evidence for the association of *Candida humicola* with tussock roots. In the previous survey (di Menna 1958) this species was found in the soil samples from tussock root zones but not in samples taken from between the tussocks, and in the present work (Table 3) it made up 50% of the yeasts isolated from the washings of tussock roots. This species has not been found on or near the roots of introduced pasture plants and was not found in the tussock-grassland soils under introduced pasture or crops. It is the only yeast of tussock-grassland soils that is necessarily affected by cultivation and replacement of the original cover.

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BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

XV. BACTERIA OF TWO CULTIVATED SOILS

By J. D. Stout, Soil Bureau, Department of Scientific and Industrial Research, Wellington

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Summary

The bacterial flora of two New Zealand soils under native tussock, introduced pasture, and cultivation for crops is described. Where the change to pasture and cultivation is very recent, there is little change in bacterial numbers but there may be more frequent occurrence of leaf strains in the topsoil until the new vegetation is established. Where the change to pasture and cultivation is well established, there is an increase in numbers associated with an increased amount of organic matter in the soil and a recurrence in the pasture soil of fermenting bacteria present in well established grassland soils.

The bacterial flora of the leaves and roots of the tussock and pasture plants was studied and also the air-borne flora. The root flora is similar to the soil flora but with an increased proportion of fermenters. The leaf flora is distinct from the soil and root flora and consists predominantly of chromogenic strains. The air-borne flora, apart from sporogenous types, is similar to the leaf flora.

Introduction

In a previous paper (Stout 1958) the bacterial flora of three New Zealand native tussock-grassland soils was described. The present paper deals with an extension of this work to soils under native tussock, introduced pasture, and crops. Its purpose was to study the microbial changes following cultivation and changes in plant cover. Two soil types were investigated (Thornton 1960). One was the Taupo soil at Waiouru previously studied. This yellow-brown pumice soil is a sandy silt of medium base status. The tussock areas are covered with introduced grasses as well as the native tussocks, *Festuca novae-zelandiae* and *Poa caespitosa*. The pasture area is dominated by sweet vernal and crested dogstail with some Yorkshire fog and white clover. This pasture has been established for eighteen years and the area under crops had been cultivated for two successive years. The second soil studied was the Cass soil at Broken River which is closely related to the Tekoa soil at Bealey previously studied. This is a sandy silt of medium base status. The native tussock cover is not very dense or luxuriant and the pasture and crops areas had only been established the previous year. One distinction between the Cass and Tekoa soils is that the Cass soil has developed under native tussock whereas the Tekoa soil developed under beech forest and is consequently more strongly weathered.

Besides studying the soil microflora at Waiouru and Broken River, the flora of the tussock and pasture-grass roots, of the tussock and pasture-grass leaves, and of the air were also studied at Waiouru for comparison with the soil flora.

MATERIALS AND METHODS

A full description of the soils and sites is given by Thornton (1960).

Soil samples were collected and studied in the manner previously described (Stout 1958). Five samples were taken in the tussock areas from the I (Intertussock) Zone, i.e., between the tussock plants. Five samples were also taken from both the pasture and the crop areas. These five samples for each area were then bulked and dilution plates prepared in the normal way. The soils were sampled in spring (October 1955) and summer (January 1956).

Large blocks of the Taupo soil under tussock and pasture were brought to the laboratory, the roots, freed as far as possible from soil, dissected out and rinsed in sterile water. Dilution plates were then prepared in the normal way.

The leaves were collected from Waiouru. The tussock leaves (*Festuca novae-zelandiae*) were brown and inrolled to form a cylinder. The pasture leaves were partly green and partly brown and included one or two flat weeds. Dilution plates were prepared from a shaken suspension of the leaves in the normal way.

The air-borne flora at Waiouru was sampled by exposing dextrose-tryptone-yeast extract (DTY) agar plates on the pasture and tussock areas. Ten plates (10 cm diameter) were exposed on each area, 5 for five minutes and 5 for ten minutes. There was a slight breeze blowing at the time of exposure.

The root, leaf, and aerial floras were sampled in August 1956.

Representative strains were isolated from the soil dilution plates and the plates prepared from the roots, leaves, and air sampling, and were tested and identified in the manner previously described (Stout 1958).

RESULTS

Soil Flora

Quantitative. The plate counts of bacteria and streptomyces are given in Table 1.

Both at Waiouru and Broken River the count was lower in spring than in summer. At Broken River the tussock count appeared a little higher in spring than the crops and pasture counts, but, in the summer, pasture and tussock had similar counts. At Waiouru the pasture count was higher than the tussock count both in spring and summer and the crops count also appeared higher than the corresponding tussock count. Both at Waiouru and Broken River, streptomyces were relatively much more numerous on the crop plates.

The general similarity of the three Broken River areas (except for the streptomyces) is probably due to the fact that the changes from tussock to pasture and root crop are very recent, having taken place in the previous year, and insufficient time has elapsed for marked soil differences to develop and affect the microbial population. At Waiouru,

TABLE 1. PLATE COUNTS OF BACTERIA (B) AND STREPTOMYCES (S) ON NUTRIENT AGAR (N. AGAR) AND DTY AGAR FOR SPRING AND SUMMER, 1955-6
(1000/g wet wt of soil)

Taupo Soil
(Waiouru)

		Tussock		Pasture		Tussock		Crops	
		B	S	B	S	B	S	B	S
Spring	N. Agar	102		178		116		256	
	DTY Agar	200		312		128		442	
	Average	151	13	245	8	122	12	349	140
Summer	N. Agar	300		800					
	DTY Agar	350		1200					
	Average	325	n.c.	1000	n.c.				

Cass Soil
(Broken River)

		Tussock		Pasture		Crops	
		B	S	B	S	B	S
Spring	N. Agar	87		55		55	
	DTY Agar	172		80		72	
	Average	129	40	67	n.c.	63	300
Summer	N. Agar	916		1050			
	DTY Agar	1150		1216			
	Average	1033	58	1133	166		

n.c.—not counted

on the other hand, the pasture has been down eighteen years and two crops have been taken off the cultivated area. Correlated with this history are the differences shown by the chemical analyses, Table 2 (cf. Thornton 1960), the pasture soil in particular showing higher total carbon and nitrogen figures but a narrower C/N ratio. The available moisture also tends to increase with the establishment of pasture and the compaction of the soil (Packard 1957).

The higher incidence of streptomyces in both the crop areas may be due to a preference for more open soil conditions or may be a reflection of greater aerial spore contamination consequent upon the more open conditions associated with the reduced plant cover.

Qualitative. The bacterial flora of the two soils is listed in Table 3.

TABLE 2. CHEMICAL ANALYSES
Tu, tussock; Pa, pasture; Cr, crops

Areas	Taupo soil (Waiouru)				Cass soil (Broken River)		
	Tu	Pa	Tu	Cr	Tu	Cr	Pa
Total organic carbon %	5.6	7.2	5.0	5.9	3.1	3.0	3.7
Total nitrogen %	0.45	0.69	0.44	0.47	0.23	0.23	0.27
C/N ratio	12	10	11	13	13	13	14
Citric-acid-soluble P mg % P	13	23	16	18	11	13	14
Cation-exchange capacity	20.7				19.8		
Total exchangeable cations	10.3				7.0		

TABLE 3. BACTERIAL FLORA OF THE SOILS—TAXONOMIC DISTRIBUTION OF THE STRAINS ISOLATED (cf. Stout 1958)

Type	Chromogenesis	Glucose Metabolism	Taupo Soil (Waiouru)				Cass Soil (Broken River)			Total No. of Isolates	%
			Tu	Pa	Tu	Cr	Tu	Pa	Cr		
Pseudomonas fluorescens I	+	O	26	26	5	8	14	1	20	100	56%
Pseudomonas sp. III	—	O	13	25	10	27	13	9	20	117	
Pseudomonas sp. IV	+	O	2		2			3	3	10	
Pseudomonas sp. VI	—	O				1	1	1	4	7	
Achromobacter spp.	—	N-o	10	8		6	9	6	6	45	19%
Flavobacterium sp. I	+	O	2	4	2	1		13	1	23	
Flavobacterium sp. II	+	O	2					4	1	7	
Chromobacterium sp.	+	O					1			1	
Aeromonas sp.	+	F	1							1	5%
Aerobacter cloacae	—	F	2	2	3		8			15	
Serratia sp.	+	F	2	2						4	
Bacillus cereus and *B. mycoides*	—	F	8	10	14	6	6	16	9	69	20%
Bacillus megaterium	—	N-o	3	2		2	2	1	3	13	
Nocardia spp.	±	N-o	1	1				1		3	
Total No. of Isolates			72	80	36	51	54	55	67	415	100

Glucose Metabolism: N-o, non-oxidative, non-fermentative, no acid produced:
O, oxidative, non-fermentative—acid produced aerobically but not anaerobically;
F, fermentative—acid produced aerobically and anaerobically.

Sites: Tu, tussock; Pa, pasture; Cr, crops.

Generally the flora is similar to that previously described and the bacterial types identified were generally identical with those previously recorded (Stout 1958). As before, it is a predominantly pseudomonad flora—oxidative and proteolytic. Generally the pseudomonads constitute half the number of strains identified from each site. An exception is the pasture site at Broken River where Achromobacteriaceae and Bacillaceae were predominant. *Flavobacterium* was particularly dominant in the spring when the pasture was barely established and when there was a very low total count. This is probably due to the infiltration of the leaf flora prior to the re-establishment of the normal grassland flora following the destruction of the tussock cover.

Apart from this exception, the chief difference between the different soil types and soil sites lies in the distribution of the small group of aerogenic fermenters. Three types were identified in the Taupo soil, *Aeromonas*, *Aerobacter*, and *Serratia*, whereas only one type, *Aerobacter*, was identified in the Cass soil. In the closely related Tekoa soil both *Serratia* and *Aerobacter* were identified and it was suggested that the distribution of these types was correlated with the vigour of the plant cover and the organic content of the soil as measured by the total-carbon figure. The absence of *Serratia* at Broken River, associated with a less vigorous tussock cover and a much lower total-carbon figure than at Bealey, appears to support this generalisation.

The distribution of these fermenters also points to a difference between the different sites in each area. They occur in the tussock site at Broken River, and in the tussock and eighteen-year-old pasture sites at Waiouru. That is to say that they occur in soils with an established grassland flora and consequently a well developed root system, but not in the soil with a temporary crop, or in a young pasture, as at Broken River. For in contrast to the Broken River pasture which had only been established for a year, the Waiouru pasture had been established for eighteen years.

Root, Leaf, and Aerial Floras

Quantitative. As the chief purpose of these studies was to determine the relationship between the soil flora and the floras of related niches, no special attempt was made at quantitative estimates. However, a count of tussock leaves collected in April 1957 gave a figure of 1.0–1.2 millions/g on a wet-weight basis and studies on ryegrass/clover pasture indicate a range of from 10 million/g to 800 million/g wet weight. Air sampled with a slit sampler at the Soil Bureau Experimental Station at Taita during November 1955 gave figures of from 3 to 64 cells per cubic foot.

Generally, the position is that the root-zone population is greater than the ordinary soil population (Stout 1958); the pasture leaves have a very high population compared both with the soil and the tussock leaves; and there are relatively few air-borne cells.

Qualitative. The root, leaf, and air-borne floras of the tussock and pasture sites at Waiouru is given in Table 4.

The root flora is essentially similar to the soil flora isolated directly from soil samples. The flora is predominantly pseudomonad (61%)

TABLE 4. ROOT, LEAF, AND AIR-BORNE FLORAS. TAXONOMIC DISTRIBUTION OF THE STRAINS ISOLATED AT WAIOURU

Type	Chromogenesis	Glucose Metabolism	Root		Leaf		Air	
			Trt	Prt	Tl	Pl	Tas	Pas
Pseudomonas fluorescenas I	+	O	5	5				
Pseudomonas sp. III	—	O	6	7	3	17	4	3
Achromobacter spp.	—	N-o	3				3	4
Flavobacterium sp. I	+	O			12	26	4	
Flavobacterium sp. II	+	O				4	8	2
Flavobacterium sp. III	+	O				13	6	2
Chromobacterium sp.	+	O				13		
Aerobacter cloacae	—	F	3			1		
Serratia	+	F	1	4				
Bacillus cereus and *B. mycoides*	—	F	1	1	2	1	3	1
Bacillus polymyxa	—	F					1	
Bacillus megaterium	—	N-o					3	
Micrococcus and *Sarcina*	±	O N-o		1	1	1	6	1
Total No. of Isolates			19	18	18	76	38	13

Glucose Metabolism as for Table 3.
Trt, tussock roots; Prt, pasture grass roots; Tl, tussock leaves; Pl, pasture grass leaves; Tas, air sample over tussock area; Pas, air sample over pasture area.

with *Ps. fluorescens* conspicuous in both series of plates. There is no marked difference between the tussock and ryegrass roots although *Aerobacter* was not isolated from the latter. However, aerogenic fermenters constitute almost a quarter of the isolates, which suggests that the root zone is particularly favourable for their growth.

There is a difference between the tussock leaf flora and that of the pasture grass. The tussock leaf flora is predominantly an orange *Flavobacterium* (66%). The pasture-grass leaf flora consists principally of three chromogenic types of *Flavobacterium* (57%), of *Chromobacterium* sp. (17%), and of *Pseudomonas* sp. (23%). There was also a single strain of *Aerobacter*.

The air-borne flora consists chiefly of *Flavobacterium* (48%) and *Bacillus* spp. (17%). Apart from non-pigmented *Pseudomonas* spp., *Achromobacter*, *Bacillus cereus*, and *B. mycoides*, the types occurring on the leaves or as air-borne flora were either uncommon or unrecorded from soil. Conversely, the common root (and soil) types, e.g., *Ps. fluorescens* or the fermenters *Aerobacter* and *Serratia*, were either rare or not recorded from leaf or air. This distinction is particularly obvious with the chromogenic types. The orange, yellow, orange-red, and violet chromogens form almost two-thirds of the leaf and air-borne floras. On the other hand, these types are typically absent from the root or soil zones where the characteristic chromogens are yellow-green to blue-green or red.

A further distinction between the soil-root flora and the leaf-aerial flora is shown by the distribution of the cocci. Virtually absent from

root and soil (cf. Stout 1958) they occur regularly in leaf and aerial samples.

The taxonomic relationships of the soil, root, leaf, and air-borne floras are paralleled by their physiological characteristics. In Fig. 1 the strains isolated from these four habitats are classified according to the scheme of Hugh and Leifson previously employed (Stout 1958). This shows clearly the greater proportion of fermenters in the soil and particularly the root populations. The higher incidence of anaerogenic fermenters (Group C) in the air-borne flora as compared with the leaf flora is due to the air-borne spores of the *Bacillus* species which are not common on leaves. The physiological character of the leaf population is on the whole less stable than that of the soil and varies according to kind and condition of the leaves.

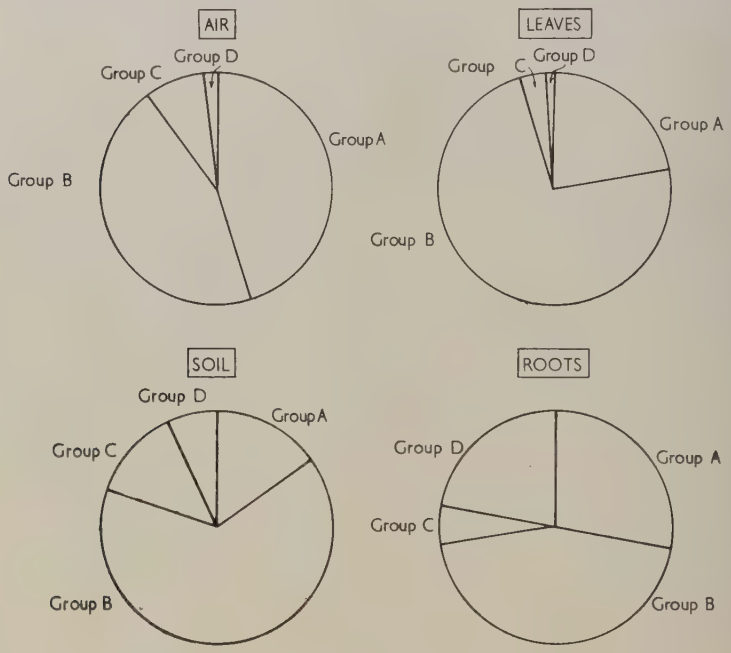

Fig. 1.—Analysis of the glucose metabolism (glucose-tryptone-bromthymol-blue) of the strains isolated from soil, roots, leaves, and the aerial plates. Group A, no acid from glucose broth; Group B, acid produced aerobically but not anaerobically; Group C, acid produced aerobically and anaerobically; Group D, acid and gas produced aerobically and anaerobically.

Discussion and Conclusions

The present paper confirms the general character of the bacterial flora previously described from the native tussock grassland soils as an essentially pseudomonad flora. It is also clear that the character of this flora is unchanged when the native tussock has been replaced by the

exotic pasture. In the transitional period, however, when the tussock has been removed and before the pasture is fully established (as at Broken River) the flora may show a greater incidence of the leaf flora bacteria such as *Flavobacterium* and also a greater proportion of spore-forming bacteria. Rovira (1956) found that an initial Gram-positive flora gave way to a Gram-negative flora in the development of the rhizosphere. This suggests that the Gram-negative flora is dependent upon the stimulus of plant growth and perhaps root excretions.

It is possible then, to distinguish three levels in the development of the soil flora. First, there are the slow-growing, non-exacting bacteria, which can be isolated with soil extract agar (Stout 1958). They are commonly Gram-positive and often spore-formers and characteristically they oxidise carbohydrates completely to carbon dioxide and water without the production of organic acids, e.g., *Nocardia* and *Bacillus megaterium*. Secondly, there are the fast-growing Gram-negative bacteria which require a richer isolation medium. They are not spore-formers, their numbers may fluctuate very markedly with changing conditions, and they oxidise carbohydrates (aerobically but not anaerobically) either with the production of organic acids, e.g., *Pseudomonas,* or completely, e.g., *Achromobacter*. Thirdly, there are the fermenters, very fast growing, and evolving hydrogen as well as carbon dioxide in the fermentation of carbohydrates. The typical genera are *Aeromonas*, *Aerobacter*, and *Serratia*.

There are two further points to be mentioned. The end products of fermentation of *Aerobacter cloacae* depend upon the oxygen tension. At high oxygen tensions there is complete oxidation of glucose, but as the oxygen tension falls so the proportion of organic acids increases (Pirt 1957). Secondly, *Pseudomonas fluorescens* has two oxygen-transport enzymes, one a cytochrome functioning at low oxygen tensions and the other a flavoprotein functioning at higher oxygen tensions (Lenhoff *et al.* 1956a, b). These facts suggest that the selective advantage of the Gram-negative bacteria in the rhizosphere is partly determined by their ability either to grow well at relatively low oxygen tensions or anaerobically. Conversely, it suggests that there is a tendency for anaerobic conditions to develop in the rhizosphere, probably as a consequence of bacterial metabolism.

It is now possible to interpret the present results in the light of this hypothesis. Table 5 summarises the results of the glucose metabolism (in glucose tryptone broth) of the strains isolated from the different soil sites, the roots, leaves, and air. The most significant fact is the incidence of the aerogenic fermenters. Most numerous in the root zone and occurring also in the tussock soils and the Waiouru pasture, they are absent from the other soil sites and are represented by a single strain of *Aerobacter* from the pasture leaves and a single strain of *Bacillus polymyxa* from the aerial plates. This strongly suggests that the aerogenic fermenters are dependent upon a well established rhizosphere. On the other hand, the temporary crops appear to favour the pseudomonads rather than the fermenters, suggesting that it is this group which reacts first to the stimulus of plant growth and that the build-up of the aerogenic fermenters is a slower process.

TABLE 5. SUMMARY OF THE GLUCOSE METABOLISM OF STRAINS FROM SOIL SITES, ROOTS, LEAVES, AND AIR
(Production of acid and gas, acid, or alkali by isolates)

	Site	Total No. of Strains Tested			% Strains		
		Alk. or n.c.	Acid	Acid and Gas	Alk. or n.c.	Acid	Acid and Gas
Cass soil (Broken River)	Tu	12	37	8	21	65	14
	Pa	10	47	0	18	82	0
	Cr	1	75	0	1	99	0
Taupo soil (Waiouru)	Tu	9	97	8	8	85	7
	Pa	7	66	4	9	86	5
	Cr	5	51	0	9	91	0
Roots (Waiouru)	Trt	4	11	4	28	50	22
	Prt	6	7	4			
Leaves (Waiouru)	Tl	10	11	0	22	77	1
	Pl	9	56	1			
Air-borne (Waiouru)	Tas	16	20	1	45	53	2
	Pas	8	8	0			

n.c., no change.

Tu, tussock; Pa, pasture; Cr, crops; Trt, tussock roots; Prt, pasture grass roots; Tl, tussock leaves; Pl, pasture grass leaves; Tas, air sample over tussock area; Pas, air sample over pasture area.

In this respect the root flora of tussock plants (*Festuca* and *Poa*) grown at the Soil Bureau Experimental Station at Taita from slips taken from Waiouru is of interest. These plants were grown in a very different soil and were examined after two years growth. Both *Pseudomonas fluorescens* and *Serratia* were absent from the flora which consisted chiefly of *Bacillus* spp. indicating that the rhizosphere flora is not simply determined by the plants, but is a function of the soil-root complex and of its past history.

One surprising feature is the very low incidence of aerogenic fermenters on leaves, for pasture leaves in particular are nutritionally rich as shown by the very high concentration of bacteria which may occur on them. Further studies, however, have shown that aerogenic fermenters may occur and be present in high numbers on ryegrass and clover pasture.

The most significant feature of the leaf flora is the very high incidence of strains possessing carotenoid pigments. These are known to have a protective role from the lethal effects of visible light in photosynthetic organisms (Sager and Zalokar 1958) and chemoheterotrophs (Kunisawa and Stanier 1958). It is also possible that these

pigmented types, the cocci, and the spores of the *Bacillus* species are more resistant to ultraviolet radiation than soil bacteria such as *Pseudomonas* and *Serratia* (cf. Zelle and Hollaender 1955). The incidence of these carotenoid pigmented types sharply distinguishes the leaf flora from the soil and root flora and confirms the view (Stout 1958) that such pigmented types isolated from soil are due to infiltration by the leaf flora.

Finally, the air-borne flora is clearly related to the leaf flora and not to the soil flora. If it is true that the soil bacteria are susceptible to visible light and/or ultraviolet radiation, this is to be expected (cf. Stanier and Cohen-Bazire 1957). The exceptions are the spore-formers whose advantages lie in the viability of this resting stage in soil assuring a more stable population than with the Gram-negative bacteria and, secondly, in the aerial distribution of the spores. The absence of such characteristic soil types as *Pseudomonas fluorescens* from leaves and from the aerial plates suggests that the distribution of the bulk of the soil flora is by migration through the soil rather than by aerial transport.

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ERRATUM

Part IV. N.Z. J. agric. Res. 1: 943-57

p. 949 Temperature Growth Range *for* 27° *read* 37°

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

XVI. NON-SYMBIOTIC NITROGEN-FIXING BACTERIA OF TWO CULTIVATED SOILS

By D. J. Ross, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 24 November 1959*)

Summary

In Taupo and Cass soils under tussock grassland in New Zealand, the numbers of bacteria producing typical colonies of the nitrogen-fixing bacterium, *Clostridium butyricum,* were found to be similar in samples taken from the base of *Festuca* tussock plants and between well-spaced *Festuca* tussock plants. Total numbers of clostridia were a little higher in soil from the base of tussock plants. The numbers of *Clostridium butyricum* in these soils were increased by the growth of introduced pastures, after preliminary ploughing and the addition of fertilisers.

Azotobacter species were not detected in these soils under tussock grassland or introduced pastures or crops. Some other aerobic bacteria isolated from the cultivated soils were shown to be without appreciable nitrogen-fixing powers.

Introduction

The anaerobic nitrogen-fixing bacterium, *Clostridium butyricum*, has previously been found in the three tussock-grassland soils examined, but *Azotobacter* species could not be detected (Ross 1958). A study is now made of two further tussock-grassland soils that had been ploughed, fertilised, and sown in crops or introduced pastures (Thornton 1960). The effects of these cultivation treatments on the numbers of non-symbiotic nitrogen-fixing bacteria are recorded in the present investigation.

Materials and Methods

Soils

A full description of the Taupo soil near Waiouru and the Cass soil at Broken River and an account of the culltivation treatments are given by Thornton (1960). Although the Taupo soil had been under introduced pasture for many years, the Taupo and Cass soils had been ploughed, fertilised, and sown with crops, and the Cass soil with introuced pasture, for little more than a year before samples for the examination for aerobic bacteria were taken. The introduced pasture under the Cass soil had been established for more than 2 years, however, when later samples were taken for the estimation of nitrogen-fixing clostridia (Table 1).

All sites had been grazed by sheep.

All soils were sampled at a depth of 2–4 in. Soils from the tussock grasslands were sampled at the base of *Festuca* tussock plants (tussock zone) and between well spaced *Festuca* tussock plants (inter-tussock zone).

Estimation of Nitrogen-fixing Clostridia

Soil samples were collected in spring, October-November 1957.

Organisms of the *Clostridium butyricum* group were grown in an N-free* glucose agar medium supplemented with an extract of Egmont brown loam and counted as before (Ross 1958). After incubation in the anaerobic jar of McIntosh and Fildes for 7 days at 24–28°c, counts were made of colonies that stained blue-purple with iodine. Gas-producing colonies were considered to be given by typical nitrogen-fixing bacteria of the *Clostridium butyricum* group. Those clostridia which produced non-aerogenic colonies, however, were regarded only tentatively as nitrogen-fixing organisms (Ross 1958).

Estimation of Nitrogen-fixing Aerobic Bacteria

Samples of all soils were collected in spring, October 1955, and samples under tussock grassland and introduced pasture were also collected in summer, February 1956.

An enrichment technique in an N-free glucose medium was used for all samples and direct plating of soil dilutions (1/10) on N-free benzoate (0.1%)-agar was also used for the summer samples. A description of the media is given by Ross (1958).

The media and methods used for the determination of the nitrogen-fixing ability of isolated bacteria were identical with those previously described (Ross 1958).

Results

Occurrence of Clostridium butyricum

In Soils under Tussock Grassland. Gas-producing bacteria of the *Clostridium butyricum* group were found in all sampling zones (column a, Table 1), but owing to variability in the counts from the tussock zone of the Taupo soil there were no significant differences between the different zones or soils. The numbers found in the Taupo soil were, however, generally higher than in the Cass soil.

The total numbers of clostridia tentatively classified as nitrogen-fixing anaerobes (column b, Table 1) were also higher in the combined zones of the Taupo soil than in the Cass soil. At both sites, the numbers of these clostridia were significantly greater, at the 5% level, in the tussock zones than in the inter-tussock zones.

In Soils under Introduced Pasture. In order to compare the numbers of anaerobic nitrogen-fixing bacteria in soils under tussock grassland and under introduced pasture, the results from the tussock and inter-tussock zones were combined to give counts which could be considered as approximately representative of the tussock-grassland soil as a whole.

In both the Taupo and Cass soils, there was an increase, significant at the 5% level, in numbers of gas-producing bacteria of the *Clostridium butyricum* group (column a, Table 2) after the growth of

* "N-free" media contained no added nitrogen compounds.

TABLE 1. COUNTS OF *Clostridium butyricum* IN TUSSOCK-GRASSLAND SOILS

Soil (Site)	Sampling Zone	No. of Samples	Soil pH	Soil Moisture, % dry wt	*Clostridium butyricum* organisms/g undried soil	
					a†	b†
Taupo soil (Waiouru)	Tussock	4	6.0	54	1,410 (100–3,550)	18,100 (11,200–24,500)
	Inter-tussock	4	6.0	53	1,020 (200–2,050)	9,200 (4,900–15,250)
Cass soil (Broken River)	Tussock	4	5.7	46	413 (250–500)	10,200 (6,200–16,500)
	Inter-tussock	4	5.9	43	388 (100–950)	6,400 (4,300–7,600)

1. †a = counts of clostridia giving gas-producing colonies.
   †b = total counts of clostridia giving gas-producing and non-aerogenic colonies.
2. Results are presented as means with the ranges of counts in parentheses.

TABLE 2. COUNTS OF *Clostridium butyricum* IN INTRODUCED-PASTURE SOILS

Soil (Site)	Vegetational Cover	No. of Samples	Soil pH	Soil Moisture, % dry wt	*Clostridium butyricum* organisms/g undried soil	
					a†	b†
Taupo soil (Waiouru)	Tussock grassland	8	6.0	53	1,020 (200–2,050)	9,200 (4,900–15,250)
	Introduced pasture	4	5.8	55	2,740 (1,750–3,550)	25,400 (18,800–41,000)
Cass soil (Broken River)	Tussock grassland	8	5.9	43	390 (100–950)	6,350 (4,250–7,600)
	Introduced pasture	4	6.3	39	660 (200–2,400)	11,700 (7,600–15,400)

1. a† = counts of clostridia giving gas-producing colonies.
   b† = total counts of clostridia giving gas-producing and non-aerogenic colonies.
2. Results are presented as means with the ranges of counts in parentheses.

introduced pastures, following preliminary ploughing and the addition of fertilisers. There was also an increase, significant at the 2% level, in the total numbers of clostridia tentatively classified as nitrogen-fixing anaerobes in these soils under pasture (column b, Table 2).

Absence of Azotobacter *Species in Soils*

Azotobacter appeared to be absent from all soils as no colonies resembling the usual species grew within the incubation period of 28 days.

Nitrogen-fixing Properties of Other Soil Bacteria

Bacteria isolated by the enrichment technique from the Taupo and Cass soils under introduced pastures were studied systematically and those showing the most vigorous growth on N-free media were examined for their nitrogen-fixing ability. These isolates did not form nodules on white clover (*Trifolium repens*) and most of their properties corresponded to those of *Agrobacterium radiobacter* (Hofer 1941) or *Pseudomonas* species. In N-free glucose liquid medium, the growth of all these bacteria was poor and was similar to that described for previous isolates from uncultivated tussock-grassland soils (Ross 1958). None of the strains showed appreciable fixation of nitrogen and there was little change in the nitrogen content of cultures in liquid media after

TABLE 3. Glucose Utilisation and Nitrogen Content of N-free Media after Growth of Some Bacteria from Soils Under Introduced Pasture

Medium	Bacterial Group	No. of Isolates	Glucose Utilised mg/ml	Nitrogen Content of Medium after Growth, µg/ml
Liquid	none	—	—	4.2† (4.0–4.4)
Liquid	*Agrobacterium radiobacter*	5	1.9 (0.8–4.6)	4.6 (4.0–5.7)
Liquid	*Pseudomonas* species	3	2.3 (1.0–4.3)	5.1 (4.2–5.9)
Solid	none	—	—	9.7† (8.3–10.5)
Solid	*Pseudomonas* species	3	—	10.8 (8.8–14.4)

1. Results are recorded as means with the ranges in parentheses; † = mean of triplicate samples of uninoculated medium.
2. Cultures were incubated for 28 days in N-free glucose liquid medium or for 14 days on N-free glucose agar. After the growth period, uninoculated liquid medium contained 8.8 mg glucose/ml.
3. Two of the isolates of *Agrobacterium radiobacter* and all isolates of *Pseudomonas* were obtained from the Cass soil.

growth for 28 days (Table 3). Any increases in nitrogen content that did occur were not correlated with the amounts of glucose utilised. Fixation by the *Pseudomonas* species was not improved by growth on agar medium.

Discussion

Although the total numbers of clostridia tentatively classified as nitrogen-fixing bacteria were found to be a little higher in the tussock than in the inter-tussock zones of Taupo and Cass soils, no significant differences were found between the numbers of typical gas-producing bacteria of the *Clostridium butyricum* group in the two zones. Previous work (Ross 1958) has shown that in Tekoa and Omarama tussock-grassland soils there were no consistent differences between the numbers of either of these groups of bacteria in the tussock and inter-tussock zones. It would, therefore, appear from the study of these four soils that conditions are, generally, little more favourable for growth of nitrogen-fixing clostridia in the environment of the roots of *Festuca* tussock plants than in soil at a distance from these plants.

The introduction of grass and clover pastures on the Taupo and Cass soils favoured the growth of nitrogen-fixing clostridia, probably due to a considerable extent to the addition of fertilisers and to the excreta of the greater numbers of grazing sheep. The 'citric-soluble' phosphate of the Taupo pasture soil was nearly double that of the uncultivated tussock-grassland soil whilst considerable amounts of super-phosphate, lime, and molybdenum had been added to the Cass soil under introduced pasture (Thornton 1960).

Although the addition of fertilisers would be expected to favour the development of *Azotobacter, Azotobacter chroococcum* could not be detected under crops or introduced pastures on these soils. As the pH of these soils was below 6.0, soil acidity probably played a large part in precluding the growth of this species (Jensen 1955). Other workers, such as Jensen (1940) in Australia, have found few acid-tolerant species of *Azotobacter* in the grassland soils of temperate countries and none could be detected in the present samples. The examination of some other aerobic bacteria showed them not to possess strong nitrogen-fixing properties although they could grow on media containing only trace amounts of nitrogen.

In uncultivated tussock-grassland soils, it is thought that non-symbiotic nitrogen-fixing bacteria add only a little to the soils' nitrogen content (Ross 1958) but over long periods of time their role could be of some importance. On cultivation of the soils, however, and the establishment of more productive pastures, their role in the nitrogen cycle becomes probably very small. In these introduced pastures, it is the symbiotic clover-*Rhizobium* association that improves the nitrogen status of the soil. Mr A. P. Druce (pers. comm.) found many clovers in the introduced pasture on the Taupo soil near Waiouru, and Bascand (1958) reported a vigorous growth of clovers on the introduced pasture on the Cass soil at Broken River. At both sites, the clovers were nodulated.

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BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

XVII. NITRIFYING ACTIVITIES OF TWO CULTIVATED SOILS

By D. J. Ross, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 24 November 1959*)

Summary

Nitrifying organisms occurred in the soils of two New Zealand tussock grasslands. The nitrifying activity of Taupo soil was less in the vicinity of the roots of *Festuca* tussock plants than in soil at a distance from these plants but little difference was found between the samples of Cass soil. These and previous results indicated that growing *Festuca* tussock plants do not stimulate the activity of nitrifying organisms in these tussock-grassland soils.

The nitrifying activity of the Taupo soil was generally greater in summer than in spring but no seasonal differences were found in the strongly acid Cass soil which had a low nitrifying potential.

In both soils, a significant increase in nitrifying activity resulted after growth of crops or introduced pastures, following preliminary ploughing and the addition of fertilisers. The activities of these cultivated tussock-grassland soils were similar to those of three other cultivated soils that were originally under forest.

Introduction

Ross (1958) found nitrifying organisms in the soils of three tussock grasslands in New Zealand. Moderate nitrifying activity was shown by Taupo soil near Waiouru and Omarama soil near Alexandra, but the activity of Tekoa soil near Bealey was low. An examination is now made of the effects of cultivation of two further sites near Waiouru and a site at Broken River, which is ten miles distant from the previous Bealey site and possesses a similar soil type. These soils, viz, Taupo soil (Waiouru) and Cass soil (Broken River), had been ploughed, fertilised, and sown in crops or introduced pastures (Thornton 1960).

A brief comparison is made with the nitrifying activities of three soils of moderate natural fertility, which were originally covered by forest. Two of these soils were, at the time of sampling, covered by introduced pasture and the other by market-garden crops.

Materials and Methods

Soils

A description of the Taupo soil near Waiouru and the Cass soil at Broken River, and a full account of the cultivation treatments, are given by Thornton (1960). The Taupo soil had been ploughed, fertilised, and sown in crops, and the Cass soil in introduced pasture, little more than a year before spring samples were taken. The Taupo soil had been under introduced pasture, however, for several years.

These two tussock-grassland soils are considered to be of low natural fertility. As based upon the ratings given by Metson (1956) for New Zealand soils, the moderately acid Taupo soil had a medium amount of total exchangeable bases, exchangeable calcium, and of 'citric-soluble' phosphate, whereas the strongly acid Cass soil had lower amounts of all these components.

The Waikanae, Judgeford, and Wharekaka soils were originally covered by forest but, at the time of sampling, were under market-garden crops or introduced pastures of grass and clover (Tables 2 and 3). The pH values of these silt loams were respectively 6.8, 6.5 and 6.3. These soils, which were of moderate natural fertility, had been fertilised with superphosphate, but the exact fertiliser treatments are not known.

Except at the market garden, all sites had been grazed by sheep.

All soils were sampled at a depth of 2–4 in. Soils from the tussock grasslands were sampled at the base of *Festuca* tussock plants (tussock zone) and between well spaced *Festuca* tussock plants (intertussock zone). Spring samples were taken in October 1955, and summer samples in February 1956. As in the previous survey (Ross 1958), this study has been undertaken to show major changes in nitrifying activity and was not designed for detailed analysis.

Media, Cultural Conditions, and Chemical Estimations

These were all identical with those described by Ross (1958). All cultures were incubated at 24°c in the dark and were examined at weekly intervals, but, unless otherwise stated, results are reported only for a 35-day incubation period. The measurement of the activity of organisms oxidising ammonia was made by estimating residual ammonia in medium A and that of organisms oxidising nitrite by measuring residual nitrite in medium B. In Tables 1-3, the amounts of ammonia and nitrite utilised are expressed as a percentage of the original concentrations.

Results

Influence of Festuca *Tussock Plants*

The nitrifying activities of the present samples of Taupo soil collected in spring and summer were greater, as a whole, when taken from the intertussock zone than from the tussock zone (Table 1). No significant differences were found between samples of Cass soil from these two zones. Results from the previous survey (Ross 1958) showed that in Tekoa and Cass soils, also, there were no consistent differences between the tussock and intertussock zones. It can be concluded, therefore, that growing *Festuca* tussock plants do not generally stimulate the activity of nitrifying organisms in these tussock-grassland soils.

Influence of Soil

It is apparent from Tables 1 and 3 that the nitrifying activity of Cass soil under tussock grassland was very low and resembled that of the similar Tekoa soil near Bealey (Ross 1958). Results with the present samples of Taupo soil were comparable with previous samples,

TABLE 1. INFLUENCE OF *Festuca* TUSSOCK PLANTS, SOILS, AND SEASON

Soil (Site)	Sampling Zone	Per cent oxidised			
		Ammonia		Nitrite	
		Spring	Summer	Spring	Summer
Taupo soil (Waiouru)	Tussock	0	56	0	0
		7	63	10	14
		32	72	99	100
		61	98	99	100
		(25)	(72)	(52)	(54)
	Inter-tussock	47	74	4	100
		69	79	16	100
		73	93	71	100
		88	96	100	100
		(69)	(86)	(48)	(100)
Cass soil (Broken River)	Tussock	0	1	6	0
		5	3	8	0
		6	10	9	1
		9	12	16	9
		(5)	(7)	(10)	(3)
	Inter-tussock	5	0	0	0
		5	0	1	4
		6	2	1	4
		21	17	1	22
		(9)	(5)	(1)	(8)

Four 1-g samples from each zone were incubated for 35 days in culture media (20 ml) containing 4.6 mg ammonium-N or 4.1 mg nitrite-N per flask. Means of the results are given in parentheses.

Variations between individual samples of the same soil were again often considerable (Table 1) and tended to confirm the irregular distribution of nitrifying organisms in tussock-grassland soils.

Influence of Season

The nitrifying activity of Cass soil under tussock grassland did not appear to be influenced by season and the activities of all samples were low (Tables 1 and 3). Ammonia was oxidised more rapidly by the Taupo soil, however, in summer than in spring but no seasonal differences were found in the oxidation of nitrite. Similar seasonal effects were recorded with the previous samples of Taupo soil (Ross 1958).

Influence of Growth of Crops

The growth of crops, after preliminary ploughing and the addition of fertilisers, resulted in a significant increase in the nitrifying activities of these soils. The increase in the Taupo soil was only slight, but a marked increase occurred in the activity of the Cass soil under crops

TABLE 2. Influence of Growth of Crops

Soil (Site)	Vegetation	Per cent oxidised	
		Ammonia	Nitrite
Taupo soil (Waiouru)	Tussock grassland (inter-tussock zone)	75 81 (78)	0 17 (9)
	Crops	93 95 (94)	0 99 (50)
Cass soil (Broken River)	Tussock grassland (inter-tussock zone)	4 8 (6)	9 15 (12)
	Crops	52 60 (56)	27 41 (34)
Waikanae soil (Lower Hutt)	Crops	47 54 (51)	100 100 (100)

Two samples of each soil were examined. The means of the results are given in parentheses.

(Table 2). The nitrifying activities of both of these cultivated soils were similar to that of the fertile Waikanae market-garden soil.

Influence of Growth of Introduced Pastures

The introduced pasture at Waiouru had been estabilshed for 18 years and resulted in a significant increase in the nitrifying potential of the Taupo soil (Table 3). Although little difference is shown in Table 3 between the activities of samples of Taupo soil after an incubation period of 35 days, samples of the introduced-pasture soil oxidised nitrite more rapidly than the uncultivated soil and oxidation was usually complete within an incubation period of 21 days.

In the recently introduced pasture at Broken River, fertilisers and DDT had been applied shortly before spring samples of Cass soil were taken. The nitrifying activity of these samples was still similar to that of the uncultivated soil, but when the summer samples were taken a few months later a marked and significant increase in nitrifying potential was apparent in the introduced-pasture soils (Table 3).

In general, similar nitrifying activities were shown by all soils sampled under introduced pastures in summer. Ammonia was oxidised at similar rates by the summer samples of Taupo soil under tussock grassland and by all soils under introduced pasture (Table 3). Taupo, Judgeford, and Wharekaka soils also oxidised nitrite at similar high rates, and the Cass soil showed moderate activity.

TABLE 3. Influence of Growth of Introduced Pastures

Soil (Site)	Vegetation	Per cent oxidised			
		Ammonia		Nitrite	
		Spring	Summer	Spring	Summer
Taupo soil (Waiouru)	Tussock grassland (inter-tussock zone)	46 90 (68)	74 100 (87)	0 100 (50)	86 100 (93)
	Introduced pasture	69 90 (80)	71 100 100 100 (93)	99 99 (99)	100 100 100 100 (100)
Cass soil (Broken River)	Tussock grassland (inter-tussock zone)	4 8 (6)	8 14 (11)	9 15 (12)	4 8 (6)
	Introduced pasture	4 24 (14)	46 48 73 73 (60)	0 6 (3)	2 18 40 75 (34)
Judgeford soil (Judgeford)	Introduced pasture	—	69 71 (70)	—	100 100 (100)
Wharekaka soil (Martinborough)	Introduced pasture	—	93 99 (96)	—	100 100 (100)

Four samples of Taupo and Cass soil collected in summer under introduced pasture, and two samples of the other soils were examined.

The means of the results are given in parentheses.

Discussion

The effects of *Festuca* tussock plants, soils, and seasons on the activities of nitrifying organisms in these present tussock-grassland soils were generally similar to those found previously (Ross 1958).

In Cass soil under tussock grassland at Broken River, the low incidence of nitrifying organisms may have been related to the low amounts of 'citric-soluble' phosphate and total exchangeable bases, such as calcium, in the soil. The strong acidity of this soil would also have been unfavourable for the activity of nitrifying organisms. The present laboratory experiments with Cass soil indicated that, in the field, there would be a shortage of nitrate available for plant growth. The results thus agreed with the conclusions of Dingwall (1956) who stated that available nitrogen was low in this area. Even when pastures

at Broken River were sown with nitrogenous fertiliser, in addition to lime, superphosphate, and sodium molybdate, symptoms of nitrogen deficiency appeared after a few months, particularly with high-fertility-demanding ryegrasses. The addition of further nitrogenous fertiliser ameliorated the unhealthy condition of these plants (Dingwall 1956). At this site, also, Bascand (1958) found that clovers established very much better than did grasses, owing undoubtedly, in part, to their lower requirements for combined available nitrogen in the soil.

In Taupo soil under tussock grassland near Waiouru, the total nitrogen content and pH were higher than those of Cass soil, and nitrification would be more rapid in the field. Present agricultural practice in developing land in this area for crops, such as turnips and cereals, and subsequently for introduced pastures does not include the addition of nitrogenous fertilisers (Moss 1956).

In both soils, the nitrifying activity was higher under crops than under the original tussock-grassland vegetation. Other investigators, such as Lyon (1922), have shown that mechanical cultivation can markedly increase the nitrifying activities of soils by improvement of their oxygen supply. Ploughing and the addition of fertilisers must have contributed considerably to the increased activity of the present soils under crops.

At Waiouru, the introduced pasture had been established for 18 years at the time of sampling. The increase in the nitrifying capacity of Taupo soil from this site was probably associated, therefore, with an improvement in soil structure resulting from the growth of the pasture herbage and with chemical factors such as the higher content of 'citric-soluble' phosphate and increased nitrogen from the growth of clovers.

In the pasture soil at Broken River, the effects of mechanical cultivation were probably very important as the pasture had been established for only one year at the time of sampling. The failure of spring samples to show any increase in activity over that of uncultivated Cass soils at the Broken River site was rather surprising, as the fertiliser treatments of both introduced pasture and crop soils with lime, nitro-lime, and superphosphate were similar, except for the addition of borax to the soil under crops and sodium molybdate to that under pasture. The insecticide, DDT, however, was applied to the pasture four months before the spring samples were taken. Although observations such as those of Bollen, Morrison, and Crowell (1954) would indicate that the concentration of DDT would be too low to restrict seriously the activity of the nitrifying organisms, it is possible that it was having some effect in this soil which was generally so unfavourable for the nitrification process. Eight months after this application of DDT, a marked increase in nitrifying potential was clearly shown by the summer samples of this introduced pasture soil.

Factors such as climate and mineral deficiencies are responsible for many of the difficulties found in establishing good quality introduced-grass pastures on the tussock-grassland soils examined in these surveys. Both surveys have shown that nitrate, available for plant growth, would also be limited in some of these soils owing to their low

rates of nitrification. However, in such a soil at Broken River, cultivation and the addition of fertilisers increased the nitrifying potential of the soil to a value comparable with those of the more naturally active tussock-grassland soils and, also, with those soils of moderate natural fertility that were under crops or pastures but were originally under forest.

Acknowledgments

I wish to thank Dr R. H. Thornton for providing samples of tussock-grassland soils, Mr H. S. Gibbs for samples of cultivated soils, and Mr W. B. Taylor, Applied Mathematics Laboratory, Department of Scientific and Industrial Research, for statistical examination of the results.

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BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

XVIII. PROTOZOA OF TWO CULTIVATED SOILS

By J. D. Stout, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 24 November 1959*)

Summary

The protozoan faunas of two soils under native tussock, introduced pasture, and cultivation for crops are described. The protozoan population is affected only where the changes brought about by a different vegetation or by cultivation result in increased organic activity and a higher bacterial population.

The protozoan fauna of tussock and pasture leaf infusions was also studied. This epiphytic fauna is distinct from the soil fauna although closely related to it. Its composition depends upon the character of the vegetation and the associated differences in bacterial populations.

Introduction

In a previous paper (Stout 1958) the protozoan fauna of three New Zealand tussock grassland soils was described. The present paper deals with an extension of this work to soils under native tussock, introduced pasture, and under crops. Its purpose was to study the microbial changes following cultivation and changes in plant cover.

Two soil types were studied (Thornton 1960). One was the Taupo soil at Waiouru previously studied. This yellow-brown pumice soil is a sandy silt of medium base status. The tussock areas are covered with introduced grasses as well as native tussocks. The pasture area is dominated by sweet vernal and crested dogstail with some Yorkshire fog and white clover. This pasture has been established for eighteen years, and the area under crops has been cultivated for two successive years. The other soil studied was the Cass soil at Broken River, which is closely related to the Tekoa soil at Bealey previously studied. This is a sandy silt of medium base status. The native tussock cover is not very dense or luxuriant and the pasture and crop areas had only been established the previous year. One distinction between the Cass and Tekoa soils is that the Cass soil has developed under native tussock, whereas the Tekoa soil developed under beech forest and is consequently more strongly weathered.

Besides studying the soil fauna, infusions of tussock leaves and pasture leaves collected at Waiouru were also studied.

Materials and Methods

A full description of the soils and sites is given by Thornton (1960).

The soil samples studied were collected in the spring (October) of 1955. There were two principal areas sampled at Waiouru, distinct

from each other and from the original tussock area sampled in the previous study (Stout 1958). One of the present areas consisted of a tussock grassland and an adjoining eighteen-year-old pasture, and the second consisted of a tussock grassland and an adjoining field which had been under crop cultivation for two years. Five samples were collected from each of the cultivated and uncultivated parts in both areas. One culture was prepared from each sample and studied in the manner previously described (Stout 1958).

At Broken River five samples were collected from undisturbed native tussock grassland and three samples each from an adjoining year-old pasture and an adjoining crop field in its first year of cultivation.

The tussock samples were collected from between the tussock plants and thus correspond to the I (Intertussock) Zone of the previous study.

The leaves were collected from Waiouru in August 1956. The tussock leaves were brown and in-rolled to form a cylinder. The pasture leaves were partly green and partly brown.

TABLE 1. NUMBER OF SPECIES IN THE TAUPO AND CASS SOIL CULTURES
R, rhizopods; C, ciliates

Taupo Soil
(Waiouru)

	Tussock		Pasture		Tussock		Crops	
	R	C	R	C	R	C	R	C
	5	5	9	9	8	8	8	11
	8	5	8	11	6	6	6	14
	7	3	12	13	8	10	12	14
	9	7	10	6	5	8	11	8
	7	6	9	10	3	6	7	15
Average	7	5	10	10	6	8	9	12

Cass Soil
(Broken River)

	Tussock		Pasture		Crops	
	R	C	R	C	R	C
	6	12	6	13	6	5
	4	8	6	9	4	9
	8	9	7	9	4	11
	5	9				
	2	8				
Average	5	9	6	10	5	8

RESULTS AND DISCUSSION

The numbers of rhizopod and ciliate species occurring in the soil cultures of the Waiouru and Broken River soils are given in Table 1 and the total number of species from each site in Tables 2 and 3.

At Broken River there is no significant difference between the faunas of the three sites, quantitatively or qualitatively. The average number of species in the cultures is relatively low as this is a yellow-brown earth of low fertility as the chemical analyses show, Table 4 (cf. Thornton 1960). It is strongly acid (pH 5.1), low in total carbon, with a C/N ratio of 14, low in available phosphate, and of medium base status. Apart from the lower total carbon and nitrogen figures, this soil is chemically very similar to the Tekoa soil at Bealey. It is less strongly weathered, the topsoil being sandy and not silty. These

TABLE 2. INCIDENCE OF RHIZOPOD SPECIES IN THE SOIL AND LEAF CULTURES
Tu, tussock; Pa, pasture; Cr, crops

Species	Soils							Leaves	
	Taupo Soil (Waiouru)				Cass Soil (Broken River)			(Waiouru)	
	Tu	Pa	Tu	Cr	Tu	Pa	Cr	Tu	Pa
Biomyxya vagans	2	4	2	4	3	1	2		
Nuclearia simplex	3	5	4	3	1	1	—		
Mayorella vespertilio	2	4	2	5	3	3	3		
Trichamoeba sp.	4	4	2	4	2	2	2	1	
Thecamoeba verrucosa	—	1	—	1	—	1	—		
Thecamoeba striata	—	—	—	—	—	—	—	1	
Amoeba	—	—	—	—	—	—	—		1
Cryptodifflugia oviformis	—	1	—	—	—	—	-		
Difflugia constricta	5	4	3	4	2	1	1		
Difflugia arcula	4	4	2	2	—	2	1		
Difflugia globulus	3	4	4	3	4	2	2		
Heleopora sp.	—	1	—	1	—	—	—		
Trinema lineare	5	5	5	5	4	3	2		
Trinema enchelys	2	4	2	1	1	1	-		
Corythion dubium	2	—	—	2	1	—	-		
Euglypha rotunda	4	5	4	5	2	2	1	1	
Euglypha ciliata	—	—	—	1	1	—	—		
Euglypha denticulata	—	1	—	2	1	—	—		
Sphenoderia dentata	—	1	—	1	—	—	—		
Total number of cultures	5	5	5	5	5	3	3	1	1
Total number of species (TS)	11	15	10	16	12	11	8	3	1
Total species-incidence (TI)	36	48	30	44	25	19	14		
Average species-incidence (TI/TS) for 5 cultures	3.4	3.2	3.0	2.7	2.3				

In species incidence at Waiouru the differences between the species are significant at 0.1%. The differences between tussock and pasture and between tussock and crops are significant at 5%. There is no significant difference between the two tussocks or between the pasture and crops.

In number of species there are no significant differences.

TABLE 3. INCIDENCE OF CILIATE SPECIES IN THE SOIL AND LEAF CULTURES
Tu, tussock; Pa, pasture; Cr, crops

Species	Soils							Leaves	
	Taupo Soil (Waiouru)				Cass Soil (Broken River)			(Waiouru)	
	Tu	Pa	Tu	Pa	Tu	Pa	Cr	Tu	Pa
Enchelys sp.	5	5	5	5	5	2	3	1	1
Spathidium sp.	—	1	1	2	1	1	—	—	1
Chaenea sp.	—	1	1	1	—	—	—		
Lacrymaria sp.	1	1	—	—	1	—	—		
Chilodonella sp.	4	3	3	4	2	3	3	1	1
Chilodonella gourandi	—	—	—	—	—	—	—	—	1
Litonotus sp.	2	3	—	3	—	—	—	—	—
Dileptus anguillula	—	4	3	4	2	1	1		
Lagynophyra sp.	—	—	—	—	1	—	—		
Pithothorax sp.	—	—	—	—	3	3	—		
Plagioplya sp.	—	—	1	—	3	3	2	—	1
Trichopelma sphagnetorum	—	3	1	5	3	3	2	—	1
Kreyella muscicola	2	4	1	3	—	—	—		
Colpoda cucullus	—	—	3	2	4	1	1	1	1
Colpoda steinii	2	3	4	3	3	2	1		
Colpoda inflata	—	2	1	2	—	—	—		
Pseudoglaucoma muscorum	3	5	3	3	2	—	2		
Tetrahymena rostrata	—	—	—	—	—	—	—	—	1
Homalogastra setosa	—	—	—	—	—	—	1		
Saprophilus muscorum	—	—	—	3	—	—	—		
Cyclidium muscicola	—	1	—	—	—	1	1		
Cohnilembus fusiformis	—	3	2	3	—	—	—		
Bryometopus pseudochilodon	—	—	—	3	2	—	—		
Uroleptus mobilis	2	3	3	1	3	2	—		
Uroleptus musculus	—	—	1	—	—	—	—		
Keronopsis muscorum	2	3	2	2	4	3	3		
Paraholosticha lichenicola	—	—	—	—	—	—	—	1	—
Oxytricha pellionella	—	2	1	4	2	2	2		
Gonostomum affine	—	—	2	5	4	3	2		
Vorticella striata	3	2	—	4	1	1	1	1	1
Tokophrya sp.	—	—	—	—	—	—	—	—	1
Total number of cultures	5	5	5	5	5	3	3	1	1
Total number of species (TS)	10	18	18	20	18	15	14	5	10
Total species-incidence (TI)	26	49	38	62	46	31	25		
Average species-incidence (TI/TS) for 5 cultures	2.6	2.7	2.1	3.1	2.6				

In species incidence at Waiouru the difference between the species are significant at 0.1%. The differences between tussock and pasture and between tussock and crops are significant at 5%. There is no significant difference between the two tussocks or between the pasture and crops.

In number of species there are no significant differences.

differences do not affect the general character of the fauna in the two soils, for much the same species are present in both, but they do affect the incidence of the ciliate species which are less numerous at Broken River than at Bealey. The rhizopods appear to be unaffected. The average number of species in the spring cultures of the two soils are R (Rhizopods) 5 : C (Ciliates) 9 for Broken River and R5 : C11 for

Bealey. The total number of species for a comparable number of cultures, is R14 : C20 for 11 Broken River cultures and R14 : C25 for the 9 intertussock-zone Bealey cultures.

At Waiouru the faunas of the two tussock sites are comparable to those previously recorded. The averages are R7 : C5 and R6 : C8 compared with the previous spring average of R8 : C7 and the three seasons average of R7 : C7 for the intertussock-zone cultures. The total number of species recorded was R12 : C22 for the present 10 cultures and R14 : C22 for the 9 previous intertussock-zone cultures. Both the pasture and the cultivated-field cultures, however, show an increase in the average number of ciliate and rhizopod species (Table 1). This appears to be due to an increased average species incidence in the case of the ciliates in the cultivated field, and to an increased

TABLE 4. CHEMICAL ANALYSES AND BACTERIAL COUNTS
Tu, tussock; Pa, pasture; Cr, crops

Areas	Taupo Soil (Waiouru)				Cass Soil (Broken River)		
	Tu	Pa	Tu	Cr	Tu	Cr	Pa
Chemical Analyses							
pH	5.8	5.6	5.8	5.3	5.1	5.2	5.2
Total Organic Carbon %	5.6	7.2	5.0	5.9	3.1	3.0	3.7
Total Nitrogen %	0.45	0.69	0.44	0.47	0.23	0.23	0.27
C/N Ratio	12	10	11	13	13	13	14
Citric acid soluble P mg % P	13	23	16	18	11	13	14
Cation Exchange Capacity	20.7				19.8		
Total Exchangeable Cations	10.3				7.0		
Bacterial Counts (1000/g wet weight)	151	245	122	349	129	67	63

number of species in the other three cases (Table 2 and 3). These differences are correlated with differences in the chemical analyses (Thornton 1960). Unlike the Broken River soils, there are marked chemical differences between the different Waiouru sites. The pasture soil has higher total carbon and nitrogen figures and a narrower C/N ratio (10). It is also higher in available phosphate. Also, the cultivated-field soil shows a small nitrogen and larger carbon increase, although the differences from the tussock soils are smaller than with the pasture, doubtless owing to the shorter period of improvement. The figures suggest that there is a better supply of nutrients in the pasture and cultivated soils than in the tussock soils, and this is supported by the bacterial counts done on these soils (Stout 1960). Unlike the Broken River counts, the Waiouru pasture and crops counts were higher than the tussock counts. It seems reasonable to associate the increased

protozoan fauna with the increase in organic matter (measured by the total carbon figure) associated with the heightened bacterial count and what is, generally, an increase in soil fertility. The previous paper showed a small increase in the ciliate fauna in the tussock zone as compared with the intertussock zone (the zone sampled in the present series) so that the more marked differences found in the present study may be reasonably attributed to changes in the organic cycle.

TABLE 5. Occurrence of the Non-protozoan Fauna in the Soils and Leaf Cultures
Tu, tussock; Pa, pasture; Cr, crops

	Soils							Leaves	
	Taupo Soil (Waiouru)				Cass Soil (Broken River)			(Waiouru)	
	Tu	Pa	Tu	Cr	Tu	Pa	Cr	Tu	Pa
Mites	—	—	—	—	—	—	×	—	—
Collembola	—	×	—	—	—	—	—	—	—
Nematodes	×	×	×	×	×	×	×	×	×
Criconemoides	×	—	×	—	×	—	—	—	—
Rotifers	×	×	×	×	×	×	—	×	×
Tardigrades	—	—	—	—	—	×	—	—	×
Enchytraeids	—	×	—	×	×	×	×	—	—

The protozoan and non-protozoan faunas of the Broken River and Waiouru soils are given in Tables 2, 3, and 5. They are similar to those previously described from Bealey and Waiouru and call for no further comment.

The faunas of the infusions of tussock and pasture leaves are also recorded in Tables 2, 3, and 5. Rotifers, nematodes, and tardigrades were present. There were few rhizopod species, but two of the amoeba species found in the infusions were not found in the soil samples. The ciliate species identified also included several species, such as *Chilodonella gourandi, Tetrahymena rostrata,* and *Tokophrya* sp. which, while they have been encountered in other soils, were not recorded in any of the present cultures of Taupo soil. One species, *Paraholosticha lichenicola,* identified in the tussock-leaves infusion, has previously been recorded only from the epiphytic lichen *Parmelia saxatilis* and the genus *Paraholosticha* appears to have a predilection for epiphytic habitats (Gellért 1955). The incidence of these species, besides a number of common soil species, indicates that the leaf-infusion fauna is distinct from the soil fauna, although the two are closely related.

The infusions of the two different types of leaves showed interesting faunal differences. The fauna of the tussock leaves consists of fewer species and those microphagous. The fauna of the pasture leaves, besides the common microphagous species, included also the histophage, *T. rostrata,* and the suctorian, *Tokophrya* sp. The implication is that the pasture-grass fauna, although comprising fewer species than the soil fauna, has periods of intense activity giving rise to a concentrated ciliate population and, consequently, conditions apt for parasitic or predatory

species. This is supported by the bacterial counts. Whereas the tussock-leaves count is not very high (10^6/g wet weight), counts of pasture leaves have reached the order of 10^9/g wet weight, a population which under suitable moisture conditions would support tremendous protozoan activity, and such activity has in fact been observed in leaf infusions from other pastures.

CONCLUSIONS

The present results confirm the essential stability of the protozoan population of a given soil and vegetation complex, such as the Taupo soil under tussock or the yellow-brown earths (Tekoa and Cass soils) under tussock. This stability is not disturbed even with a change of plant cover, provided that, as at Broken River, such a change does not cause marked differences in the soil organic cycle. Where, however, cultivation or pasture establishment do result in increased soil fertility, as at Waiouru, this is reflected by the protozoan population, partly by an increase in the number of species recorded and partly by the more frequent occurrence of species.

The epiphytic fauna of the tussock and pasture leaves is clearly distinct from the soil fauna and is dependent directly upon the character of the vegetation and its ability to support microbial activity. This is shown by the differences between the faunas of the tussock leaves and that of the pasture leaves.

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CORRIGENDA

Part VII *N.Z. J. agric. Res. 1:* 974-84.

Table 2 *add:*

In *species incidence* the difference between species is significant at 0.1%; the interactions Species × Place and Season × Place are significant at 1%.

In *number of species* there are no significant differences.

Table 3 *add:*

In *species incidence* the differences between species and between places are significant at 0.1%. The interaction Species × Place is significant at 0.1%.

The differences in *numbers of species* are significant at 0.1%.

Table 4—Rhizopods. *For* Taupo *read* Taupo Soil.

A NOTE ON THE OCCURRENCE OF NON-SYMBIOTIC NITROGEN-FIXING BACTERIA IN SOME INTRODUCED-PASTURE SOILS

By D. J. Ross, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 30 November 1959*)

Summary

Azotobacter species were not detected in five soils, of medium natural fertility, which were originally under forest but which had been under introduced-grass pastures for several years.

Counts were made of *Clostridium butyricum* in two of these soils under grass and clover pastures. The numbers of these anaerobic bacteria were low and the amounts of nitrogen fixed by them in pastures would be very small compared with those fixed by the clover-*Rhizobium* association.

Introduction

In previous studies, an examination has been made of the occurrence of non-symbiotic nitrogen-fixing bacteria in soils in New Zealand under tussock grassland and in introduced pastures derived from them (Ross 1958b, 1960). *Azotobacter* species were not detected in any of the soils. Although the pH of the Omarama soil, pH 6.8, was favourable for its growth, most of the soils were probably too acid to support the growth of *Azotobacter chroococcum,* the usual species found in the soils of temperate countries. Anaerobic bacteria of the *Clostridium butyricum* group were found in these tussock-grassland soils and their numbers increased on the addition of fertilisers and the growth of pastures of introduced grasses and clovers.

Many of the more fertile soils now under pasture in New Zealand were originally under forest. Although it has generally been assumed that non-symbiotic nitrogen-fixing bacteria play little part in the nitrogen economy of these grass and clover pastures, studies of their numbers in these soils do not appear to have been previously reported. An examination of some of these fertile soils has, therefore, been made and a comparison drawn with results from the tussock-grassland soils. At one of the sites, near Marton, a remnant of the original forest bordered the pasture and the soil from this forest has also been examined for non-symbiotic nitrogen-fixing bacteria.

Materials and Methods

Soils

All soils were obtained from areas in the North Island of New Zealand. They were originally covered by forest or scrub but for more than fifty years had been mainly under pastures of introduced grasses and clovers. The Wharekaka soil near Martinborough was taken from a site normally used for crops but at the time of sampling was also under pasture. A brief description of the soils is recorded in Table 1

and 2 and further details are given by New Zealand Soil Bureau (1954). Chemical analyses were made by methods described by Metson (1956).

The Korokoro soil near Wellington was of low to medium, and the other soils of medium, natural fertility. These five soils, with the possible exception of the Korokoro soil which is described by di Menna (1957), had been fertilised with superphosphate but the amounts used were not known.

All sites had been grazed by sheep.

All soils were sampled at a depth of 2–4 in.

TABLE 1. Chemical Analyses of Soils

Soil (Site)	Vegetation	Org. C (%)	Total N (%)	C/N	P* (mg %)	Total exch. bases (me. %)	Lab. No.
Horotiu sandy loam (Claudelands)	Pasture	4.1	0.34	12	—	9.7	7244
Kiwitea silt loam (Marton)	Pasture	8.0	0.65	12	5	16.3	6973
	Forest	11.7	0.96	12	9	44.4	6940B
Wharekaka silt loam (Martinborough)	Pasture	4.0	0.30	13	7	8.1	959A
Judgeford silt loam (Judgeford)	Pasture	6.0	0.34	18	4	5.4	4260A
Korokoro silt loam (Judgeford)	Pasture	5.3	0.34	16	3	6.4	6486A

* Soluble in 1% citric acid.

Estimation of Azotobacter

Direct plating of soil dilutions (1/10) on N-free* benzoate (0.1%) agar (B), and N-free mannitol (1.0%)-agar (M), were used for some samples and an enrichment technique in an N-free glucose medium (G) for others (Table 2). A description of the media is given by Ross (1958b). Duplicate examinations were made of each sample and cultures were incubated at 24°c for at least 28 days.

Estimation of Nitrogen-fixing Clostridia

Organisms of the *Clostridium butyricum* group were grown in an N-free glucose medium supplemented with an extract of Egmont brown loam (Ross 1958b). The medium was poured at 45°c unless otherwise stated. After incubation in the anaerobic jar of McIntosh and Fildes

* N-free media contained no added nitrogen compounds.

for 7 days at 24°–28°c, counts were made of gas-producing colonies that stained blue-purple with iodine.

Results

Absence of Azotobacter *Species*

Azotobacter appeared to be absent from all soils as no colonies resembling the usual species grew within the incubation period of 28 days. In general, the present soils may have been too acid to favour the growth of *Azotobacter chroococcum* but factors other than the acidity of the soil would appear to have been responsible for the absence of this organism in the Judgeford and Wharekaka soils and in Kiwitea soil under forest. The content of phosphorus soluble in citric acid was low in some of the soils but was at a medium level in the forest soil. Although the total exchangeable bases were low in some of these pasture soils they were very high in the soil under forest and the concentration of cations should have been sufficient for growth of this organism.

Occurrence of Clostridium butyricum

In the tussock-grassland soils previously studied, the numbers of typical gas-producing colonies of *Clostridium butyricum* varied with the soil and the mean counts per gramme of fresh soil were 390 in Cass soil and 1,210 in Taupo soil. When these soils were fertilised and sown in introduced grasses and clovers, the mean counts per gramme of fresh soil rose to 660 in Cass soil and 2,740 in Taupo soil.

The numbers of *Clostridium butyricum* were determined in two of the present soils under pasture. The mean counts obtained per gramme of fresh soil were 380 in the Horotiu soil and 290 in the Kiwitea soil (Table 2). These counts are similar to those reported by Swaby (1939) for some soils in Victoria, Australia. They are significantly lower than those found previously in the Cass and Taupo pasture soils derived from tussock grassland and it is, therefore, unlikely that there is a simple relationship between the soil fertility of pastures and the numbers of these nitrogen-fixing organisms.

Although the numbers of *Clostridium butyricum* appeared to be lower in Kiwitea soil under forest than under pasture, there was little difference between them when they were calculated on dry weight of soil, as the mean counts were then 590 per gramme of pasture soil and 520 per gramme of forest soil. The agar medium used for counting clostridia in the Kiwitea soil was poured at 100°c. It is thus possible that the counts reported in Table 2 for this soil were slightly lower than the numbers occurring in the field as it has been shown (Ross 1958a) that growth of these organisms may be retarded under these cultural conditions.

Conclusions

Although the numbers of samples studied in this and previous investigations have been small it would appear that *Azotobacter* species are not widely present in soils under native tussock grassland or

TABLE 2. COUNTS OF *Azotobacter* AND *Clostridium butyricum*

Soil (Site)	Vegetation	Dates of Sampling	Soil pH	Soil Moisture, % dry wt	Counts of *Azotobacter*			Counts of *Clostridium butyricum*	
					Media[1]	No. of samples	No. organisms /gramme fresh soil	No. of samples	No. organisms/ gramme fresh soil[2]
Horotiu sandy loam (Claudelands)	Grass/clover pasture	Feb. 1957	6.1 (5.7–6.4)	10 (9–11)	BM	5	Nil	5	450 (280–720)
		Mar. 1957	6.1 (6.1–6.2)	33 (29–36)	BM	5	Nil	5	440 (290–620)
		Jul. 1957	6.1 (5.9–6.1)	28 (20–34)	BM	5	Nil	5	240 (160–380)
Kiwitea silt loam (Marton)	Grass/clover pasture	Feb. 1957	6.1 (5.8–6.2)	38 (29–48)	BM	5	Nil		
		May 1957	5.7 (5.3–6.1)	51 (46–57)	BM	5	Nil	5	290[3] (140–500)
	Broadleaf-podocarp forest	Feb. 1957	6.8 (6.6–7.0)	70 (56–83)	BM	5	Nil		
		May 1957	6.6 (6.2–7.0)	71 (52–90)	BM	5	Nil	5	150[3] (100–320)
Wharekaka silt loam (Martinborough)	Grass/clover pasture	Feb. 1956	6.5	14	BG	2	Nil	—	—
Judgeford silt loam (Judgeford)	Grass/clover pasture	Feb. 1956	6.3	23	BG	2	Nil	—	—
Korokoro silt loam (Judgeford)	Browntop/sweet vernal pasture	Sep. 1956	6.0 (5.9–6.1)		G	5	Nil	—	—

Results are presented as means with ranges in parentheses.

1. B = N-free benzoate agar.
   M = N-free mannitol agar.
   G = N-free glucose medium (enrichment technique).
2. Only gas-producing organisms were counted.
3. Organisms counted after growth in the medium poured at 100°C.

introduced pastures in New Zealand. The acidity of some of these soils would be unfavourable for their growth. Additional factors, however, must be responsible for the absence of *Azotobacter* in many of the soils, but the nature of these factors has not been determined.

The numbers of *Clostridium butyricum* were lower in the present pasture soils than in some of the soils examined previously under tussock grassland or introduced pastures derived from them. However, the numbers were of the same order of magnitude in all soils examined. The contribution of these anaerobes to the nitrogen reserves of the soil would be dependent upon their metabolic activity in the soil but, even under the most favourable conditions, the amounts of nitrogen fixed would be considerably less than one pound per acre per year. In fertile grass and clover pastures, hundreds of pounds of nitrogen are fixed per acre per year. The present results thus support the statement of Walker (1956) that the amounts of nitrogen fixed by non-symbiotic micro-organisms in New Zealand pasture soils are probably insignificant in comparison with those added by the clover-*Rhizobium* association.

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A NOTE ON FUNGI ISOLATED FROM PLANTS

H. Jacks, Massey Agricultural College,
Palmerston North

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In a previous note (Jacks 1958) the occurrence was recorded of *Aspergillus, Chaetomium, Epicoccum, Macrosporium, Phoma,* and *Stemphylium* species on a sample of stained wool. Work here reported was carried out to ascertain the incidence of fungi on plants.

Seeds were obtained of the following:

Grasses: I. Paspalum (P. *dilatatum* Poir.), II. Italian ryegrass (*Lolium multiflorum* Lam.) III. Perennial ryegrass (*L. perenne* L.), IV. Crested dogstail (*Cynosurus cristatus* L.), V. Chewings fescue (*Festuca rubra* L. var *commutata* Gand.), VI. Yorkshire fog (*Holcus lanatus* L.), VII. Sweet vernal (*Anthoxanthum odoratum* L.), VIII. Browntop (*Agrostis tenuis.* Sibth.), IX. Timothy (*Phleum pratense* L.), X. Cocksfoot (*Dactylis glomerata* L.), XI. Meadow foxtail (*Alopecurus pratensis* L.), XII. Short-rotation ryegrass (*L. multiflorum* x *L. perenne* L.)

Legumes: XIII. Lucerne (*Medicago sativa* Link.), XIV. Clustered clover (*Trifolium glomeratum* L.), XV. Subterranean clover (*T. subterraneum* L.), XVI. Red clover (*T. pratense* L.).

Weeds: XVII. Spurry (*Spergula arvensis* L.), XVIII. Ribgrass (*Plantago lanceolata* L.), XIX. Fathen (*Chenopodium album* L.), XX. Fennel (*Foeniculum vulgare* Mill.), XXI. Scotch thistle (*Cirsium lanceolatum* (L) Hill.), XXII. Dock (*Rumex* sp.), XXIII. Oxtongue (*Helminthia echioides* Gaertn.), XXIV. Creeping buttercup (*Ranunculus repens* L.), XXV. Hairgrass (*Vulpia bromoides* (L) Gray), XXVI. Sheeps sorrel (*Rumex acetosella* L.), XXVII. Oxeye daisy (*Chrysanthemum leucanthemum* L.), XXVIII. Broadleaved plantain (*Plantago major* L.), XXIX. Seed of chou moellier (*Brassica oleracea* L.), was also used.

Sowings were made in Manawatu silt loam placed in 5 in. pots, five replications for each type of seed. After sowing, the soil in each pot was moistened with 250 ml of a spore suspension of *Chaetomium, Epicoccum,* and *Phoma* species in sterile distilled water. Pots were subsequently tray watered. The mean glasshouse temperature during the test was 64°F ± 16°F. Plants were cut twice at 4 in. from the soil surface, and sprayed with 1 acre–inch simulated rainfall after each cut. Ten isolations were made from each pot, one month after the second cut. Isolates were taken from apparently intact plant tissues, discoloured portions, and necrotic lesions, and from dead blades and leaves; these were repeatedly washed in sterile distilled water and plated on potato dextrose agar.

In general, percentages of the total number of isolations made show in descending order of incidence the frequency of fungous genera

TABLE 1. INCIDENCE OF FUNGOUS GENERA CALCULATED FROM THE TOTAL NUMBER OF ISOLATES PER PLANT

Numbers refer to plants listed in the text.
Letters refer to genera listed in the text.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
I	35	19		4	12	4		6	10		2	2				2			2							
II	59	2	11	4	11			7				2			2			2								
III	22	22	17	16	3	2	2	3		2		2	5								2	2				
IV	24	12	4	2		18	22	2	2	2	10	2														
V	21	14	25	7	7	3		7	3										3			7				
VI	27	18	3	7	9	11		3	3	2	7	2			3			2		2						
VII	27	16	10	5	8	8			8		5	3	5		3											
VIII	28	8	18			6		2	4		16	1	4		2			4		2	4					
IX	23	30	7	27		5			2		2	2						3								
X	29	7	13	8	11	5	3										8		3			2				
XI	11	33	37		7						4		4				4									
XII	56				19					6	12		6													
XIII	17	31	17		14	3		3		6	6				3				3							
XIV	24	10	10	5	13	10	3	3		5	5		3	5				3		3						
XV	8	27	27	5	8	3				3	5			3		3		8								
XVI	50	5				5	10	5			10					5		5	5							
XVII	7	24	19	7	9	9	5		4	10		2							2		2		2			
XVIII	32	24	8		8			4	4							12				8						
XIX	23	10	14	5	14	7	5		5		2			2	14											
XX	7	38	7	10	3	3	14		7			3												7		
XXI	10	31	30															3				6			3	
XXII	33	12	6	14	7	8		6	3		5	1			3			1								
XXIII	47		11	24		10	3														3					
XXIV	25	5	10	40				5					5					5							5	
XXV	16	15	5	2	15	5		12	10	2	3	8					5					3				
XXVI	21	27	15		12	3	3			3	9			3			3									
XXVII	34	3	13	4		6		3			18	5					4									
XXVIII	6	3	25	34		12					12										9				6	3
XXIX	3	14	16		8	5	5	8	22		3	5				3	3		3							

occurring on the plants used in this experiment: A. *Penicillium* 25%, B. *Stemphylium* 16%, C. *Epicoccum* 13%, D. *Fusarium* 8%, E. *Chaetomium* 7%, F. *Mucor* 5%, G. *Aspergillus* 3%, H. Cladosporium 3%, I. *Phoma* 3%, J. *Macrosporium* 1%, K. *Botyrtis* 5%, L. *Alternaria* 1%, and at less than 1%—M. *Geotrichum,* N. *Pullularia,* O. *Hormiscium,* P. *Monotospora,* Q. *Bacteria,* R. *Rhizoctonia,* S. *Hormodendron,* T. *Torula,* U. *Trichoderma,* V. *Helminthosporium,* W. *Oospora,* X. *Sporotrichum,* Y. *Thielavia,* and Z. *Dactylella.*

The fungi previously isolated from wool gave 43% of the total number of isolations. Other fungi recorded in the above experiment may have colonised the plants by way of spore movement from the air and soil.

In Table 1 is given the incidence of the main fungous genera on various plants. To simplify the table, plants are listed in their numerical order and the organisms are indicated by letters.

The occurrence of these fungi on plants confirms the suggestion made previously (Jacks 1958), that wool could be stained when sheep were resting, especially when conditions are favourable for the prolific growth of these organisms. Fungi may have a detrimental effect on wool (White *et al.* 1950) especially when the epicuticle has been damaged, and staining may be caused when the fleece is damp, temperatures are high, and the animal is suffering from some physiological disorder.

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THE INSECT COMPLEX OF SOWN PASTURES IN THE NORTH ISLAND

X. INSECTS REVEALED IN STUDIES OF SOIL, TURF, AND DUNG

By R. A. Cumber, Entomology Division, Department of Scientific and Industrial Research, Palmerston North

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Summary

The insect species taken during 1957 and 1958 spring and summer surveys of soil, turf, and dung at widely scattered North Island localities involving the majority of pasture types, are discussed with regard to their identity, distribution, and possible economic importance. This information is complementary to that obtained during a sweep-sampling survey carried out during the summer of 1957.

Introduction

During 1957 and 1958, a study was made of the insects associated with the various pasture types in widely scattered North Island districts. The information obtained from summer sweep-net sampling has already been presented (Cumber 1958, 1959a, b, c. d, e, f, 1960; Cumber and Harrison 1959). The present account concerns those species taken during the summer and spring examinations of soil, turf, and dung. The Collembola have not been considered although their importance is fully realised.

Distribution maps are given for 4 species, a black dot indicating the presence of a species in contrast to a ring where sampling failed to reveal it.

Many of the species encountered have already been dealt with in previous papers in the series. This is indicated by an asterisk following the name of the species.

Results

SECTION I. DUNG EXAMINATIONS

Examinations of cattle dung were carried out at the 56 detailed survey stations during the summer of 1957. At each site, 12 dung pats were examined, a selection of dung of various ages being studied where such was available. In addition to the dung itself, the surface of the soil beneath and to a depth of several inches was studied.

Order **Hemiptera**

He. 1. *Dictyotus caenosus* (Westwood) (Pentatomidae)*

As many as 15 adults and nymphs were taken beneath single dry dung pats at Victoria Valley, Kaitaia 16.1.57, Kerikeri 18.1.57, and Whananaki 21.1.57. Dry, hot conditions prevailed at the time and the bugs were doubtless taking shelter and advantage of the relatively succulent adjacent non-grazed herbage.

Order **Diptera**

D. 1. *Sarcophaga milleri* J. et T. (Calliphoridae)*

This species was found breeding in cattle dung at the great majority of sampling sites (Fig. 1). Larvae occurred in very considerable numbers in the newer pats, and puparia were readily obtained from the soil beneath older ones. A selection of these puparia was taken at various localities. The flies which emerged belonged only to this species. In three cases a percentage of the puparia was parasitised by a hymenopterous insect *Ericydnus?* sp. (Hy. 38)*.

Adults of *S. milleri* from the one locality usually vary in size quite considerably. The puparia taken at Woodhill however were all very small, and thin and papery. The adults which emerged, like the sample obtained during sweep-netting, comprised very small individuals. Whether a small local race was involved, or whether unfavourable breeding conditions preceding the particular generation was responsible, has not been determined.

Order **Coleoptera**

Co. 42. *Rhytisternus miser* Chaud. (Carabidae)

Distribution

This species is of widespread occurrence in the North Island. It was taken in small numbers at Kerikeri 18.1.57 and Gisborne-Tiniroto 12.2.57.

Habits and Economic Significance

These are not known, but it probably plays a minor beneficial role in pastures.

Co. 43. *Clivina rugithorax* Putz. (Carabidae)

Distribution

This species is widely distributed in the North Island. Small numbers of typical specimens and in addition several examples of what are apparently variants of the same species (varying degree and extents of red colouring of the elytra) were taken at Victoria Valley, Kaitaia 16.1.57, Whangaroa 17.1.57, Ruahaka 21.1.57, and Dargaville 21.1.57.

Habits and Economic Significance

Muggeridge (1939) describes serious damage to germinating maize grains and to ripe strawberries caused by this insect which has American relatives accused of similar non-carnivorous habits. Whether or not this insect is in addition carnivorous, as are most of the Carabidae, is not known. It is not considered to be important in pastures.

Co. 64. *Anchomenus submetallicus* White (Carabidae)

Distribution

This endemic species is widely distributed in New Zealand. It was taken in small numbers in association with dung only twice, at sites

near Kaitaia on 16.1.57. It is recorded as coming to light at Paiaka (Manawatu) from September till February (Cumber 1953).

Habits and Economic Significance

Nothing is known of the habits of this species which probably plays a minor beneficial role in pastures.

Co. 46. *Thyreocephalus chloropterus* (Erichs.) (Staphylinidae)

Distribution

This apparently introduced species was taken in small numbers from beneath dung pats at Whangaroa 17.1.57 and Maraekakaho 11.2.57.

Habits and Economic Significance

These are not known, but it possibly plays a minor beneficial predaceous role in pastures.

Co. 65. *Leptacinus* sp. (Staphylinidae)

Small numbers were taken at Napier on 11.2.57. The remarks on the previous species apply here.

Co. 33. *Lacon variabilis* Cand. (Elateridae)*

Pupae and adults were taken on and in the soil beneath old dry dung pats at Waikohu on 13.2.57.

A small number of undetermined elaterid larvae were taken from beneath dung at Tuakau 7.3.57, Maraekakaho 11.2.57, and Norsewood 26.3.57.

Co. 11. *Hyperodes griseus* Hust. (Argentine Stem Weevil) (Curculionidae)*

This was taken in small numbers from beneath dung at Dannevirke on 26.3.57.

Co. 65. *Saprosites rugosus* Richards (Scarabaeidae)

Distribution

Richards (1959) records this species from "Auckland; Mt. Maunganui; Kaipara Harbour; Gisborne; Russell; Waiheke Island; Okauia; Brooklands." In the present survey a small number of adults were taken from beneath dung at Ruakaka 21.1.57, Dargaville 22.1.57, and Whakatane 14.2.57.

Habits and Economic Significance

These beetles are generally regarded as dung feeders and of no importance in pastures, although the allied genus *Aphodius* contains serious pasture pests.

Co. 62. *Costelytra zealandica* (White) (Grass Grub) (Scarabaeidae)

Distribution

This species is widely distributed in New Zealand. In the present survey larvae (1–5) were taken from the soil beneath dung at Waikohu 13.2.57, Tarata 2.4.57, Manaia, Taranaki 2.4.57, and Masterton 25.3.57.

Habits and Economic Significance

This is the common grass grub which is currently regarded as the most damaging pasture species in New Zealand. A considerable volume of literature on its biology and methods of control is available.

Order **Hymenoptera**

Hy. 38. *Ericydnus*? sp. (Encyrtidae)*

This species was obtained on 3 occasions from puparia of *Sarcophaga milleri*. In the first case (collections at Woodhill 23.1.57) 28 puparia were held. Two failed to emerge, 19 flies emerged, and the remaining 7 puparia produced 20 parasites. In the second case (Whangaroa, 17.1.57) 2 puparia yielded flies, the third producing 16 parasites. In the third case (Whananaki 21.1.57) 15 puparia were held. Five failed to emerge, 9 flies emerged, and a single puparium produced 6 parasites.

Hy. 37. *Ponera eduardi* Forel (Formicidae)*

A small number of workers were taken beneath drier dung pats at Maraekakaho on 11.2.57.

Order **Orthoptera**

Or. 2. *Lissotrachelus maoricus* (Walk.) (Gryllidae)*

A small number of nymphs and adults were taken sheltering beneath drier dung pats at Victoria Valley, Kaitaia 16.1.57, and Warkworth 22.1.57.

Or. 6. *Acheta commodus* (Walk.) (Field Cricket) (Gryllidae)*

Adults and nymphs, sometimes in moderate numbers, were taken from beneath the drier dung pats at Victoria Valley, Kaitaia 16.1.57, Whangaroa 17.1.57, Kerikeri 18.1.57, Paeroa 5.3.57, Gisborne 23.1.57, Tiniroto 12.2.57, and Foxton 4.4.57. Dung affords excellent shelter from predators during the daylight hours.

Order **Lepidoptera**

Le. 11. *Pseudaletia separata* (Walk.) (Introduced Army Worm) (Noctuidae)*

Small numbers of larvae were taken sheltering beneath the older dung at Victoria Valley, Kaitaia 16.1.57, Dargaville 22.1.57, and Whakatane 14.2.57.

Le. 22. *Agrotis ypsilon* Rott. (Greasy Cutworm) (Noctuidae)

Distribution

According to Hudson (1928) this species is of worldwide distribution and occurs throughout New Zealand. In the present survey larvae and pupae were taken from beneath dung at Victoria Valley, Kaitaia 16.1.57, Kerikeri 18.1.57, Puketona 18.1.57, Punakitere 19.1.57, Dargaville 22.1.57, Ruakaka 21.1.57, Whananaki 21.1.57, and Woodhill 23.1.57.

Habits and Economic Significance

This species is well known as a serious pest in private gardens and in many commercial crops, the larvae attacking roots and stems about and below ground level. The moths are present from October till April, but like the larvae are active mainly during the hours of darkness. In some cases as many as 10 larvae per dung pat were taken. The highest numbers were encountered where conditions were dry and grazing was close. In the immediate vicinity of the dung the grass normally avoided by stock plus the shelter provided by the dung produced favourable conditions for the insect.

Although this species is doubtless of some importance in local areas, it is not considered to be of general importance in North Island pastures.

Undetermined noctuid materials—pupae and pupal skins—and several larvae of doubtful identity occurred in small numbers at a few additional sites.

In a small number of instances slugs were taken in moderate numbers. The season was an unusually dry one, however, which would tend to minimise their importance.

SECTION II. SOIL AND TURF EXAMINATIONS

The information presented here was obtained during 3 surveys.

1. Soil and turf examinations were made on the spot at the detailed survey stations during the summer (January-April) 1957. In the initial stages samples were collected for laboratory examination, but owing to the additional work they entailed and the relatively little information which they supplied, these collections were discontinued.
2. Examinations of soil and turf were carried out at the detailed survey stations during the spring (September-November) of 1957. Turf samples were also collected and studied in the laboratory. It was felt that this spring soil and turf survey would supplement very considerably that picture obtained during the summer investigations.
3. During the spring of 1958 (September) field examinations were carried out at 104 widely separated stations sited on the various soil and pasture types occurring in North Auckland.

Much of the work was carried out in conjunction with that of Todd (1959) during his studies on the biology and distribution of the black beetle (*Heteronychus sanctae-helenae* Blanch.).

At each sampling site 4 randomly spaced 8-inch-square, spade spits approximately 8 inches in depth were studied, and single additional spits were taken where close laboratory examinations were carried out. Numbers of insects taken refer to totals for the 4–5 spade-spit samples unless otherwise indicated. During the surveys more than 900 spade-spit samples from some 150 localities widely distributed in the North Island were studied.

Order **HEMIPTERA**

He. 39. *Rhopalosiphum padi* (L.) (Aphididae)*

A small number of specimens were taken at Puketona 2.10.57, Wellsford 7.10.57, Pukekohe 6.11.57, and Inglewood 8.11.57.

He. 40. *Geoica lucifuga* (Zehn.) (Aphididae)*

This species was taken in small numbers at Waiare 19.9.58, and Gisborne and Gisborne-Tiniroto 4.11.57. It is probably widespread in the North Island. At Waiare it was taken from roots of *Microlaena stipoides* R. Br., the ant *Monomorium antarcticum* (White) being in attendance.

He. 60. Undetermined (Pseudococcidae)

Distribution

This species was taken on 54 occasions at widely separated North Island localities (Fig. 2).

Habits and Economic Significance

This insect is associated with the roots of a number of grasses, and in drier friable conditions may occur in moderate populations. Its importance is difficult to evaluate.

Fig. 1

Fig. 2

Fig. 1.—Distribution map for the calliphorid *Sarcophaga milleri* (D. 1).

Fig. 2.—Distribution map for the pseudococcid. Undetermined (He. 60).

He. 58. *Melampsalta* spp. (Cicadidae)

Distribution

Melampsalta spp. are widely distributed in New Zealand. The species have not yet been separated in their nymphal stages. It seems probable that the one mainly concerned in pastures is *M. muta* (F.) an occasional specimen of our largest species *M. cingulata* F. also being taken. In the present survey, nymphs (1–5) were taken at Whangaroa 22.9.58, Maitahi 26.1.58, Kaipara flats 18.9.58, Warkworth 18.9.58, and Norsewood 26.3.57, 26.11.57.

Habits and Economic Significance

Nymphs are present during all months of the year (Cumber 1952). No populations of significant proportions were taken during the surveys, and it is unlikely that they exist in the normal pastures, for eggs are laid and overwinter in coarse seed-head stems.

He. 18. *Halticus tibialis* Reuter (Miridae)*

A single adult was taken at Warkworth on 22.1.57.

He. 26. *Deraeocoris maoricus* Woodward (Miridae)*

A single adult was taken at Warkworth on 22.1.57.

He. 9. *Nysius huttoni* F. B. White (Lygaeidae)*

Single adults were taken at Dargaville 3.10.57 and Carterton 28.11.57.

He. 1. *Dictyotus caenosus* (Westwood) (Pentatomidae)*

A single adult was taken at Whananaki on 21.1.57.

He. 22. *Nezara viridula* (L.) (Green Vegetable Bug) (Pentatomidae)*

A single adult was taken at Whananaki on 21.1.57.

Order **DIPTERA**

A small number of miscellaneous dipterous larvae were encountered. Single tipulid larvae ("leather-jackets") were taken at only 3 localities namely, Pakaraka 19.9.58, Pukemiro 7.11.57, and Hunterville 20.11.57.

Order **COLEOPTERA**

Co. 64. *Anchomenus submetallicus* White (Carabidae)

(See also Section I.) Single adults were taken at Dargaville 22.1.57 and Paeroa 6.11.57.

Co. 43. *Clivina rugithorax* Putz. (Carabidae)

(See also Section I.) Adults (1–3) were taken at Victoria Valley, Kaitaia 16.1.57, Kerikeri 2.10.57, Ohaewai 3.10.57, Dargaville 3.10.57, Tangiteroria 25.9.57, Waimamaku 26.9.58, Ruakaka 21.5.57, Helensville 7.10.57, and Mangaweka 20.11.57.

Co. 57. *Cyclothorax rotundicollis* (White) (Carabidae)

Single specimens of this endemic species which was originally described from the Bay of Islands were taken at Maramarua 6.11.57 and Marton 18.11.57.

Co. 42. *Rhytisternus miser* Chaud. (Carabidae)

(See also Section I.) Single adults were taken at Maitahi 26.1.58 and Ruawai 29.1.58.

In addition to the above species a small number of carabid larvae were taken at 7 localities.

Co. 65. *Leptacinus* sp. (Staphylinidae)

(See also Section I.) A single adult was taken at Eketahuna on 28.11.57.

Co. 67. *Corymbites antipodus* Cand. (Elateridae)

A single adult was taken at Hunterville on 20.11.57.

Miscellaneous elaterid larvae were taken at 21 widely separated localities during the months of September, October, November, and February. The greatest number taken at any one sampling site was 3.

Co. 8. *Cyphon* sp. (Helodidae)*
A single adult was taken at Victoria Valley, Kaitaia, on 16.1.57.

Co. 25. *Eucolaspis* sp. (Chrysomelidae)*
A single adult was taken at Norsewood on 26.11.57.

Co. 7. *Catoptes* spp. (Curculionidae)*

Distribution

In the present survey some 150 specimens (mainly larvae) were taken at 38 sites (Fig. 3), the greatest number (20) being taken at Mataraua, Northland on 26.9.58.

Habits and Economic Significance

Several species of *Catoptes* are concerned, and it would appear that increasing attention should be given to this widely distributed group of species which must be considered as important pasture pests. Larvae were taken in September, October, November, January, February, and March, pupae in November and February, adults in November.

Co. 40. *Graphognathus leucoloma* (Boheman) (White-fringed Beetle) (Curculionidae)

Distribution

In a previous paper (Cumber 1959b) reference is made to larval attack in North Auckland areas with *Pantomorus godmanni* Crotch. as the suspected species. It has now been established that *G. leucoloma* is concerned here.

G. leucoloma is native to South America where it is widely distributed in the southern parts. It has been established in the south-west of the U.S. and Australia for some years. In New Zealand the species is apparently present in two main North Island areas namely, the

southern portions of North Auckland and Hawke's Bay. In the present survey it was taken at Wellsford 18.9.58, site 1, 24 larvae; site 2, 6 larvae; Woodhill 7.10.57, 2 larvae; 29.9.58, 18 larvae; and Maraekakaho 4.11.57, 3 larvae.

Habits and Economic Significance

Metcalf *et al.* (1951) indicate that the larval stages of this insect are known to damage more than 380 kinds of plants. Overwintering is in the larval stages, the flightless adults emerging in spring to feed on the nearby plants. Females produce parthenogenetically and are very prolific, a single individual sometimes laying in excess of 1,800 eggs which hatch in mid-summer. Adults live several months and apart from accidental transportation may crawl up to three quarters of a mile.

The full-grown larva is about the size of the mature grass grub, but it is fatter and does not possess the clearly defined legs of that grub.

Where heavy infestations were observed near Wellsford there was a noticeable thinning-out of clovers and grasses and a tendency for flat weeds to become prominent. It seems that considerable trouble from this species may be expected in the future—perhaps in crops rather than in permanent mixed pastures however—and that it deserves study and formulation of control measures. DDT dusts which have been cultivated into the soil have shown promise of control in the U.S.

Co. 11. *Hyperodes griseus* Hust. (Argentine Stem Weevil) (Curculionidae) *

(See also Section I.) Single adults were taken at Tarata 8.11.57 and Marton 20.11.57.

Co. 66. *Saprosites rugosus* Richards (Scarabaeidae)

(See also Section I.) Adults (1–3) were taken at Fairburn 23.9.58, Kaikohe 26.9.52, Ruakaka 7.10.57, Dargaville 22.1.57, Whakatane 14.2.57, and Wellsford 7.10.57.

Co. 62. *Costelytra zealandica* (White) (Grass Grub) (Scarabaeidae)

(See also Section I.) This species was taken at 21 widely separated localities in the North Island (Fig. 4). The majority of the infestations encountered (mainly larvae) were light however, the greatest number taken being 10. Only 3 other samples showed a total in excess of 5 grubs for the 4–5 spade-spit samples.

Co. 68. *Heteronychus sanctae-helenae* Blanch. Black Beetle. (Scarabaeidae)

This species was taken at a number of sampling sites in northern areas. A full account of the distribution and biology of this species is given by Todd (1959).

Co. 69. *Pyronota festiva* F. Tea-tree Beetle (Scarabaeidae)

Distribution

This species is widely distributed in the North and South Islands,

Fig. 3.—Disribution map for the curculionids *Catoptes* spp. (Co. 7).

Fig. 4.—Distribution map for the scarabaeid *Costelytra zealandica* (Co. 62).

In the present survey larvae were taken at Ahipara 22.9.58, Whangaroa 17.1.57, Tarata 8.11.57, pupae at Eketahuna 28.11.57, and adults at Rotorua 6.11.57. At no sampling site were more than 3 individuals taken.

Habits and Economic Significance

No detailed studies have been made of the life history of this species, which, it is supposed, may be similar to that of the common grass grub. It is apparently of some importance under local specialised soil conditions (Cumber and Cowie 1954), but not of importance generally in the North Island.

Order **Hymenoptera**

Hy. 37. *Ponera eduardi* Forel (Formicidae)*

(See also Section I.) A small number of workers and a queen were taken at Victoria Valley, Kaitaia 30.9.57, Whananaki 7.10.57, Woodhill 7.10.57, Maramarua 6.11.57, Tarata 8.11.57, Manaia (Taranaki) 8.11.57, Sanson 18.11.57, and Carterton 28.11.57.

Hy 47. *Monomorium antarcticum* (White) (Formicidae)*

A few workers and a queen were taken at Victoria Valley, Kaitaia 30.9.57, Whangaroa 22.9.58, Waiare 19.9.58, Tarata 8.11.57, and Carterton 28.11.57.

Hy. 88. *Pheidole yarrensis* Forel (Formicidae)

This species has been recorded from New Zealand only recently (Cumber in press), although it is known to have been present for many years. It was taken at Whangaroa on 17.11.57 (2 soldiers).

Order **ORTHOPTERA**

Or. 2. *Lissotrachelus maoricus* (Walk.) (Gryllidae)*

(See also Section I.) A few nymphs were taken at Tarata 8.11.57 and Carterton 28.11.57.

Orthopterous eggs probably belonging to *Acheta commodus* occurred in samples from Whangaroa 30.9.57 and Pukekohe 6.11.57.

Order **LEPIDOPTERA**

Le. 23. *Oxycanus* sp. (Subterranean Grass Caterpillar) (Hepialidae)

Distribution

Oxycanus (*Porina*) spp. are present throughout New Zealand. In the present survey a small number of larvae were taken at Dargaville 22.1.57 and Eketahuna 28.11.57.

Habits and Economic Significance

Some species of *Oxycanus* are particularly injurious to pastures especially in southern areas, and have received considerable attention in the past. It seems that in relatively restricted areas—especially of hill country—a moderate degree of damage occurs in the North Island.

Le. 1. *Coleophora alcyonipennella?* Kollar (Clover Case-bearer) (Coleophoridae)*

A small number of larval cases were taken at Napier on 4.11.57.

Le. 4. *Crambus flexuosellus* Dbld. (Crambidae)*

This species was taken at Wellsford 7.10.57 (1 pupa) and Carterton 28.11.57 (4 larvae). Their association with moss at the latter site may have some significance. This species is very commonly seen on the wing in southern parts of the North Island and the low number of soil and turf detections is surprising. The insect possibly breeds in less-disturbed areas of pasture.

Le. 22. *Agrotis ypsilon* Rott. (Noctuidae)

(See also Section I.) Several larvae were taken at Ruakaka on 21.1.57.

Undetermined noctuid materials in the form of larvae, pupae and empty pupal skins were taken at 16 widely separated localities, but only in small numbers. There were in additon a number of miscellaneous undetermined lepidopterous larvae.

MOLLUSCA

Several species of slugs were encountered at some 14 localities. On some occasions moderate populations were present such as occurred at

Te Kuiti (7.11.57) where 9 were taken from a single spade-spit sample. Although the overall damage in established pastures may not be very great, slugs may well present a serious problem where pasture establishment is involved.

Two tiny introduced snail species were taken in relatively small numbers at 5 sites. They are of no importance in pastures.

Discussion

Dung may be regarded in the light of its potentiality as a breeding medium, or its value as a protective covering for insect life. For example the fly *Sarcophaga milleri* is associated with dung mainly because it utilises its substance for the rearing of its larvae. It is true however that adults ingest the liquids and that the fully developed larvae which leave the manure and pupate in the soil beneath receive protection from the covering pat. But it would appear that the majority of the insects associated with dung are there for the protection which it affords. The relatively hard sun-baked dung pats, anchored and partly raised by the grass growth, form a very fine protection for nocturnal species such as crickets and noctuid larvae. The lush untouched growth of adjacent cover enhances the protective value.

The majority of the species associated with dung are probably not of great importance to pastures generally. There are a number of predacious carabid and staphylinid species which possibly play a beneficial role, and there are many casual associates of minor importance. One should, however, recognise the potential fecundity of most insect species, and the relative importance which must be placed on even a limited number of satisfactory survival sites.

The fly *S. milleri* mentioned above is one of the commonest species associated with pastures, and it is often confused with other species such as the house fly and the biting stable fly. Its close association with dung and adjacent herbage makes it an immediate suspect in the cases of transmission of intestinal diseases.

The field cricket *Acheta commodus* is very commonly associated more especially with the older dung pats in northern areas where it often assumes damaging proportions. The importance of protection from birds given by ground cracks has often been stressed in studies on this insect. It is probable that the importance of undisturbed dung is also fully realised by most farmers in the affected areas, and that the appropriate measures are taken. Whether or not dung plays any part in the protection of overwintering eggs is not known.

Turning now to the insects taken during the soil and turf examinations, we find, as was expected, a number of species which have either not shown up, or shown up rather poorly, in the summer sweep programme. Table 1 compares the numbers of species in the different orders (excluding the Collembola) taken in summer sweep-netting, in summer dung examinations, and in spring and summer soil examinations, and shows the vast superiority of summer sweep-sampling as a means of determining the insect fauna.

TABLE 1. NUMBER OF SPECIES TAKEN IN SAMPLINGS

Order	Summer Sweep-netting	Summer and Spring Turf and Soil	Summer Dung
Hemiptera	47	10	1
Diptera	68	3	1
Coleoptera	32	15	9
Hymenoptera	84	3	2
Orthoptera	5	4	2
Lepidoptera	20	4	2
Psocoptera	7	—	—
Thysanoptera	12	—	—
Neuroptera	1	—	—

The soil coccids deserve some comment. They are commonly present throughout the North Island in the better-drained pastures, and sometimes occur under moderately damp conditions, no doubt taking advantage of their water-repelling mealy properties. Nothing is known of their relative mobility in the soil. It may well be very reduced with a dependence on their food coming to them (as roots) rather than their going in search of food. The levels of population make it unlikely that nutrition drainage is very great, but there are in addition the possibilities of toxin production and virus transmission which may influence their importance.

The same remarks apply here as in the section on dung in respect of the Carabidae and Staphylinidae.

Wireworms occurred fairly regularly but nowhere in significant numbers, so that it is suspected that while local sporadic higher-level populations may exist from time to time, they are of little overall importance.

New Zealand is, by overseas standards, singularly free from damaging forage and fodder-crop curculionids, and unwelcome establishments from time to time are inevitable. The present work has indicated two species, one endemic and the other introduced, which must receive increasing attention. The endemic one, *Catoptes* (several species appear to be involved) is of widespread occurrence in New Zealand, and as indicated above is often present in considerable numbers. Night sweep-netting at the times of adult emergences would almost certainly produce considerable numbers. This species doubtless has its preferences amongst the pasture plant species and its full effects are likely to be noted only where purer stands are developed. Its overall effects must be considerable, however, and it appears to survive in areas treated with DDT for grass-grub control. There is an urgent need for systematic and biological studies on the *Catoptes* group of weevils in New Zealand. The introduced species, which is just establishing itself as an important and damaging species, is the American white-fringed beetle *Graphognathus leucoloma*. As in *Catoptes* spp., damage is more likely to be marked in pure stands, although it is likely to become widely established in pastures.

Hyperodes griseus (the Argentine Stem Weevil) has been discussed previously in this series. It is now receiving increasing attention in New Zealand and a substantial volume of information is anticipated.

The Scarabaeidae include the best-known pests of pastures in New Zealand. The common grass grub *Costelytra zealandica,* although turning up at a number of widely scattered localities, was nowhere encountered in excessive numbers, or on the same scale as *Catoptes* spp. It is known, of course, that relatively sporadic local and severely damaging outbreaks do occur in some areas, but the general picture provided by the present survey is one of overall minor importance in comparison with the situation in the South Island. *Pyronota festiva* as was anticipated, occurred in a small number of localities at low numbers. This species is also known to reach damaging proportions in local North Island areas. *Saprosites rugosus* was taken in small numbers especially in northern areas.

The writer is of the opinion that natural microbial control of scarabaeid pests plays a much greater part under conditions of permanent pasture than has generally been supposed in New Zealand in the past, and that fluctuating population levels are a compatible phenomenon.

No high lepidopterous populations were encountered. *Oxycanus* occurred in surprisingly low numbers, although as indicated previously, high populations are known to occur in some areas.

The present investigation would indicate that high priority should be given to increasing our knowledge of the part which weevils play in the pasture economy of this country.

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EFFECT OF APPLE AND PEAR BOX LINERS ON FRUIT IN COOL-STORAGE

By C. A. S. Padfield, Fruit Research Division, Department of Scientific and Industrial Research, Havelock North

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Summary

Waxed paper and polyethylene case-liners partially reduced wilting and sometimes restricted weight loss of both apples and pears during cool storage. Polyethylene bag liners, both sealed and unsealed, eliminated wilting and further restricted weight loss. Jonathan Spot was controlled by storing apples in polyethylene bags. Advantages in these respects were offset by increased losses due to other physiological disorders, and, in some instances, by "off" odours and flavours. The use of polyethylene sheet- or bag-liners is not recommended.

Introduction

During cool storage some varieties of apples and pears are particularly susceptible to wilting, which detracts from their appearance and market acceptability. The most seriously affected New Zealand-grown apple varieties are Delicious, Golden Delicious, and Sturmer Pippin, and the pears P. Barry, Winter Cole, and Winter Nelis. It has already been shown (Padfield 1957) that the inclusion of an inner waxed-paper liner in packed cases improved the appearance and reduced weight loss in stored Winter Cole pears. This procedure is cheap and has now become standard commercial practice for this variety, but these waxed liners do not completely prevent wilting.

Since the introduction of plastic films, attempts have been made to use such materials as case liners. For example, polyethylene film made up into bag type liners has been widely adopted for pear storage in North America (Hardenburg, Schomer, and Uota 1958). Several workers (including Hardenburg and Siegelman 1957) have reported the possible hazard of carbon-dioxide injury when fruit is stored in polyethylene. Padfield and Mandeno (1953) controlled Jonathan Spot in controlled atmosphere ("gas") storage. As polyethylene film is known to restrict gas movement, it was considered possible that Jonathan apples held in sealed polyethylene bags might not develop this disorder.

Experimental

In 1956, 1957, and 1958, trials were conducted using wilt-susceptible varieties of apple and pear. Boxes with standard corrugated cardboard liners only were used as checks, while all other boxes, in addition, included one of the following: waxed-paper sheet-liners, polyethylene sheet-liners (1.5 mils), polyethylene bag-liners of the same thickness, either sealed or unsealed. Liners were supplied to selected growers and were placed in cases during normal packing operations, but to ensure that sealing was uniform, this operation was undertaken by the author.

It is possible that treatments shown as folded and not sealed could have been as effectively sealed as those that were sealed either by heat or rubber rings. All bags had a generous overlap and when these were folded and held in position by the box-lid, a seal could have been effected.

Samples of three or four bushels were stored at the temperature appropriate for the variety. Each variety was held until small samples from the check treatment, withdrawn at monthly intervals, showed some form of deterioration. On removal from cool store all fruit was held before examination for a further 14 days at 60-70°F. Seals were broken immediately on removal from cool store, except where otherwise shown. Description of disorders (Padfield 1954) and the method of recording were similar to those previously described (Padfield 1959). Net weights of fruit were recorded at the end of each experiment, but it was not possible to weigh fruit before storage. No analyses of atmospheres within bag liners were made during the experiments.

RESULTS

Apples

Delicious (Table 1)

Waxed paper liners and polyethylene sheet liners apparently did not influence weight loss of Delicious apples. There was some increase in scald with both polyethylene liners and bags and an increase in brown heart with the latter.

Golden Delicious (Table 2)

In 1956 and 1957 both waxed paper and polyethylene liners reduced wilting while polyethylene bags eliminated all such symptoms from fruit of this variety.

In 1957, significant breakdown occurred in fruit of advanced maturity stored in polyethylene bags and it is possible that this was due to the modified atmospheres within the bags. Rots were reduced in fruit of both picks stored in bags. These bags appeared to reduce scald considerably in the first pick. Less weight appeared to be lost by fruit in polyethylene bags, and, except for increased internal breakdown in the second pick, bag liners appeared generally beneficial for this variety.

Sturmer Pippin (Table 3)

With this variety in both years waxed paper and polyethylene liners reduced, and polyethylene bags eliminated, wilting but in one year there was an increase of other disorders with use of polyethylene. This fruit also suffered from "off" odour and flavour. Symptoms similar to brown heart were recorded in fruit held in both sheet- and bag-liners.

Jonathan (Table 4)

In both years, control of Jonathan Spot and a slight reduction of wilt were obtained by storing fruit in polyethylene bags, but this advantage was offset by the development of either more serious internal breakdown and core flush or superficial scald.

TABLE 1. CONDITION OF DELICIOUS APPLES AFTER COOL STORAGE IN VARIOUS CASE LINERS

Picked 11 April. Examined 26 to 29 November 1957

Type of Liner	Internal Breakdown %	Core Flush %	Fungus %	Mouldy Core %	Superficial Scald %	Wilt %	Brown Heart %	Sound		Mean Net Weight per Bushel lb
								Totally %	Commercially %	
Corrugated cardboard	3	2	9	3	19	4	0	66	87	39.1
+ Waxed sheet	9	1	14	7	18	1	0	65	74	39.1
+ Polyethylene sheet	10	1	18	13	34	1	1	49	57	39.3
+ Polyethylene bag, folded	17	4	10	9	38	1	12	34	50	41.6

TABLE 2. CONDITION OF GOLDEN DELICIOUS APPLES AFTER COOL STORAGE IN VARIOUS CASE LINERS

Type of Liner	Internal Breakdown %	Core Flush %	Fungus %	Wilt %	Superficial Scald %	Bitter Pit %	Sound		Mean Net Weight per Bushel lb
							Totally %	Commercially %	
Picked 10 April. Examined 26 to 30 September 1956									
Corrugated cardboard	0	0	7	43	9	0	48	73	35.9
+ Waxed Sheet	0	0	6	26	18	0	59	76	35.8
+ Polyethylene bag, folded	0	0	2	0	12	0	86	94	37.4
+ Polyethylene bag, heat-sealed, seal broken on removal from cool store	0	0	5	0	4	0	91	94	36.8
Picked 6 March. Examined 9 to 16 September 1957									
Corrugated cardboard	1	6	13	39	80	7	9	68	34.3
+ Waxed sheet	2	3	15	11	75	6	18	73	34.9
+ Polyethylene sheet	0	2	14	13	58	8	29	78	34.9
+ Polyethylene bag, folded	1	2	5	0	28	9	61	93	36.3
Picked 27 March. Examined 17 to 19 September 1957									
Corrugated cardboard	6	1	21	19	6	5	54	70	35.2
+ Waxed sheet	1	0	18	6	18	4	63	79	35.5
+ Polyethylene sheet	1	0	15	3	14	2	72	84	36.0
+ Polyethylene bag, folded	24	2	6	0	2	4	70	76	37.4

TABLE 3. CONDITION OF STURMER PIPPIN APPLES AFTER COOL STORAGE IN VARIOUS CASE LINERS

Type of Liner	Internal Breakdown %	Core Flush %	Fungus %	Wilt %	Superficial Scald %	Bitter Bit %	Brown Heart %	Sound		Mean Net Weight per Bushel lb
								Totally %	Commercially %	
Picked 9 April. Examined 24 to 29 October 1956										
Corrugated cardboard	21	13	15	19	4	0	0	39	72	
+ Waxed sheet	25	20	16	5	15	0	0	41	76	
+ Polyethylene bag, folded	19	13	3	0	2	0	0	67	89	
+ Polyethylene bag, heat-sealed, seal broken on removal from cool store	14	1	2	0	1	0	0	83	94	
+ Polyethylene bag, heat-sealed, seal not broken until 14 days after removal from cool store	13	0	1	0	0	0	0	87	96	
Picked 9 April. Examined 13 to 20 November 1957										
Corrugated cardboard	47	46	25	31	64	2	0	3	23	38.6
+ Waxed sheet	41	59	20	18	68	2	0	5	29	38.1
+ Polyethylene sheet	63	77	40	7	65	3	10	2	12	38.6
+ Polyethylene bag, folded	41	25	10	0	6	0	12	42	73	39.7

TABLE 4. CONDITION OF JONATHAN APPLES AFTER COOL STORAGE IN VARIOUS CASE LINERS

Type of Liner	Internal Breakdown %	Core Flush %	Fungus %	Wilt %	Jonathan Spot %	Lenticel Spot %	Brown Heart %	Superficial Scald %	Sound		Mean Net Weight per Bushel lb
									Totally %	Commercially %	
Picked 11 March. Examined 8 to 11 July 1957											
Corrugated cardboard	26	12	4	7	21	9	0	0	45	80	38.0
+ Polyethylene bag, folded	84	76	1	0	0	0	1	0	13	25	38.2
+ Polyethylene bag, rubber-ring sealed	82	75	1	0	0	0	2	1	14	30	37.8
+ Polyethylene bag, heat sealed	88	78	2	0	0	0	2	1	9	22	39.0
Picked 7 March. Examined 16 to 18 June 1958											
Corrugated cardboard	1	0	1	8	4	0	0	0	86	98	
+Polyethylene bag, folded, no holes	2	0	0	0	0	0	0	19	80	94	
+ (Polyethylene bag, folded, with 2 × $\frac{1}{4}$ in. holes	1	0	1	0	0	0	0	21	78	92	

Pears

P. Barry (Table 5)

All treatments reduced wilting and weight loss, while polyethylene bag liners eliminated wilting. In all other treatments wilting was confined to the stalk end of the fruit.

TABLE 5. CONDITION OF P. BARRY PEARS AFTER COOL STORAGE IN VARIOUS CASE LINERS

Type of Liner	Fungus %	Wilt %	Sound		Mean Net Weight per Bushel lb
			Totally %	Commercially %	
Picked 1 April. Examined 26 and 27 November 1956					
Corrugated cardboard	2	58	41	97	35.2
+ Waxed sheet	3	19	78	97	36.4
+ Polyethylene bag, folded	7	0	93	93	38.5
+ Polyethylene bag, heat-sealed	10	0	90	90	37.9
Picked 21 March. Examined 22 to 25 November 1957					
Corrugated cardboard	23	24	54	74	36.5
+ Waxed sheet	14	9	78	86	37.3
+ Polyethylene sheet	16	3	80	84	37.8
+ Polyethylene bag, folded	27	0	73	73	37.5

Winter Cole (Table 6)

Wilting was eliminated only by polyethylene bags. Where sheet liners were used wilting was restricted to pears against case ends, particularly in the top and bottom layers. Similarly bag-liners restricted weight loss to a greater extent than either type of sheet liner. These advantages were offset by development of assumed carbon dioxide injury, which took the form of longitudinal cortical cracking.

TABLE 6. CONDITION OF WINTER COLE PEARS AFTER COOL STORAGE IN VARIOUS CASE LINERS
Picked 15 March, Examined 18 to 21 August 1957

Type of Liner	Core Breakdown %	Fungus %	Carbon Dioxide Injury %	Wilt %	Sound		Mean Net Weight per Bushel lb
					Totally %	Commercially %	
Corrugated cardboard	2	5	0	54	38	57	32.7
+ Waxed sheet	0	2	0	22	75	91	34.4
+ Polyethylene sheet	0	3	0	19	79	91	34.1
+ Polyethylene bag, folded	0	2	10	0	88	92	35.8

Winter Nelis (Table 7)

In 1956, waxed liners reduced visible wilting, but did not restrict weight loss. Sealed bags appeared to reduce core breakdown while both sealed and unsealed bags eliminated wilt and restricted weight loss. In fruit stored in bag-liners, core breakdown was accompanied by browning, this discoloration being evident in some fruits before core breakdown developed. It is probable that this was also a form of carbon dioxide injury.

In 1957, fruit for this experiment was harvested in over-ripe condition and this probably explains the early development of rots. Storage life was so short that wilt hardly had time to develop.

TABLE 7. CONDITION OF WINTER NELIS AFTER COOL STORAGE IN VARIOUS CASE LINERS

Type of Liner	Core Breakdown %	Fungus %	Wilt %	Sound		Mean Net Weight per Bushel lb
				Totally %	Commercially %	
Picked 9 April. Examined 30 September to 3 October 1956						
Corrugated cardboard	42	13	42	31	50	37.0
+ Waxed sheet	43	13	11	44	50	37.0
+ Polyethylene bag, folded	38	8	0	62	62	38.2
+ Polyethylene bag, heat-sealed	14	7	0	81	81	38.2
Picked 24 April. Examined 1 to 3 July 1957						
Corrugated cardboard	0	45	3	49	54	38.2
+ Waxed sheet	0	34	0	66	66	38.2
+ Polyethylene sheet	1	36	1	63	64	38.6
+ Polyethylene bag	1	28	0	71	71	38.9

CONCLUSIONS

With most varieties tested the use of various case-liners reduced both wilt and weight loss, polyethylene bags being the most effective. Polyethylene bag-liners with certain varieties sometimes reduced but often increased the incidence of other storage disorders and their use for storage of apples and pears in New Zealand cannot be recommended. Polyethylene sheet liners had little advantage over waxed paper liners.

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A NOTE ON THE MECHANISM OF THE CONVERSION OF CAROTENE TO VITAMIN A IN THE ANIMAL BODY

By W. A. McGillivray,* Biochemistry Department, Massey College, Palmerston North

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Summary

Based on the results of experiments in which aqueous dispersions of carotenoid pigments have been administered intravenously to rats, it is suggested that the mechanism of conversion of carotenes to vitamin A in the animal body involves a rapid, non-specific oxidation of the carotene molecule to retinene which is then reduced to vitamin A alcohol. The initial breakdown of the carotene molecule would seem to involve an unsaturated lipid-coupled oxidation with haematin compounds of the blood or tissues as catalysts.

Introduction

It is almost 30 years since Moore (1930) reported his classical experiments demonstrating the provitamin A activity of β-carotene. During this period the reaction involved has been studied by many workers in attempts to elucidate the mechanism of the transformation in the animal body. The process would appear to be a simple one, originally thought of as a hydrolytic fission of the central double bond of the β-carotene molecule but now more generally regarded as a breakdown of the provitamin to two molecules (or possibly only one molecule) of retinene (vitamin A aldehyde) which is then rapidly reduced to vitamin A alcohol (Moore 1957). If this latter postulate is correct —and all available evidence points in this direction—it is only the nature of the initial oxidative breakdown of the carotene molecule which remains obscure since there is ample evidence of the widespread occurrence in animal tissues of an enzyme system capable of converting retinene rapidly and efficiently to vitamin A (Glover, Goodwin, and Morton 1948).

However, so far all attempts to effect the *in vitro* conversion of carotene to vitamin A, as a first step in the isolation of the enzyme systems involved, particularly in the initial oxidation steps, have failed or at best have produced equivocal results. To date the tissues most extensively studied have been the liver, formerly thought to be the site of conversion of carotene to vitamin A, and the wall of the intestine, now firmly established as the main site of conversion following oral dosing and the organ therefore most likely to show high enzymic activity. In general the enzyme sought, or claimed to have been demonstrated, has been a relatively specific "carotenase" or lipoxidase which would induce oxidation either by direct attack on the central double bond of the β-carotene molecule or in a stepwise manner from one end of the molecule, the former reaction yielding two molecules.

* Present address: The Dairy Research Institute (N.Z.), Palmerston North.

and the latter one molecule, of retinene from each molecule of β-carotene oxidised.

Recent work in this laboratory (McGillivray and Worker 1957, 1958; Worker 1959) has widened the scope of conversion mechanism studies since, from the results obtained using intravenously administered aqueous dispersions of various carotenoids, it appears that the ability to initiate a suitable oxidation of the carotene molecule is not limited to the wall of the intestine but is probably common to a great many, if not all, tissues including blood. While this could indicate a widely distributed specific enzyme system or systems capable of oxidising a range of carotenoids (those containing a β-ionone ring yielding, in the process, retinene) it seems more likely that the breakdown of the carotenoid molecule involves some more non-specific type of oxidation. Glover and Redfearn (1954) have in fact presented evidence supporting a type of β-oxidation but there is little evidence regarding the mechanism involved.

From a consideration of possible oxidation reactions and of the available data regarding the conversion of orally and parenterally administered carotene to vitamin A, there seems strong evidence for postulating that the initial reaction is coupled with an unsaturated lipid oxidation involving catalysis by haematin compounds. In support of this suggestion it has already been noted that the initial stages at least of the conversion of intravenously administered carotene to vitamin A occur mainly in the blood stream (McGillivray and Worker 1958). The catalytic activity of haematin compounds such as haemoglobin of the blood and myoglobin of other tissues has been extensively investigated by Tappel (1954, 1955a, b) and would seem to be sufficiently rapid to explain the almost instantaneous appearance of vitamin A following injection of carotene. Previously unreported work from this laboratory has shown that the efficiency of conversion of intravenously administered carotene (as judged by liver reserves of vitamin A) may be increased up to threefold by incorporating in the aqueous dispersion of β-carotene, an unsaturated lipid such as methyl linoleate. It is also significant that haematin catalysis is only effective in a heterogeneous system (Haurowitz, Schwerin, and Yenson 1941). The aqueous dispersions of carotene which have been used in this investigation are of this type and when injected intravenously are subject to rapid breakdown and conversion to vitamin A. However, within about 15 minutes of injection, the carotene remaining in the blood (and that taken up by the tissues) is much more stable and does not undergo the same type of breakdown (McGillivray and Worker 1958). It has now been shown that the dispersing agent (Tween 40) is hydrolysed fairly rapidly after injection and in the process the dispersed carotene becomes associated with blood proteins, mainly globulins. Under these conditions there is no longer a heterogeneous oil-water system, the catalytic activity of haematin compounds is inhibited, and the carotene, like the carotenoids naturally present in a similar protein-bound form in, for example, bovine blood, acquires a surprising degree of stability.

Thus, as a possible mechanism for the conversion of intravenously administered carotene to vitamin A, it is suggested that immediately

following injection there is a rapid, unsaturated lipid-coupled oxidation of the carotene molecule, the haemoglobin of the blood acting as the main haematin catalyst. As a free-radical reaction, the initial point or points of attack will be determined to some extent at least by the electronic configurations of the carotenoid molecules and those carotenoids containing one or more β-ionine rings would be expected to produce at least one molecule of retinene. Because of its β-methyl group this retinene may be protected to some extent from further oxidation (c.f. β-methyl substituted acids, Carter *et al.* 1939), but more likely it may be very rapidly removed from the oxidising environment by the tissues where it is reduced to vitamin A. Tocopherol which, in high concentrations, has been shown to inhibit the conversion of carotene to vitamin A may therefore be regarded merely as playing its normal anti-oxidant role in a free-radical, unsaturated lipid oxidation.

Following oral administration it is suggested that the mechanism of conversion is similar, myoglobin of the tissue replacing haemoglobin of the blood as the haematin catalyst. The wall of the intestine also contains the enzyme systems necessary for the reduction of retinene and the esterification of the resultant vitamin A.

The findings on which these suggestions are based will be published in detail later.

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SOIL STUDIES USING SWEET VERNAL TO ASSESS ELEMENT AVAILABILITY

IV. PHOSPHORUS

By N. Wells and W. M. H. Saunders, Soil Bureau, Department of Scientific and Industrial Research, Wellington

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Summary

The phosphorus status of New Zealand topsoils is surveyed in terms of total, organic, and inorganic phosphorus fractions, solubility of the inorganic phosphorus in N H_2SO_4 and phosphorus content of the grass, sweet vernal (*Anthoxanthum odoratum*). Use is made of the genetic soil classification to show how the plant and soil phosphorus values are influenced by the nature and degree of the soil-forming processes.

The total phosphorus contents of mineral topsoils at early stages of weathering reflect the nature of the parent rock in that red-brown loams from basalt and yellow-brown loams from andesite contain larger amounts of phosphorus than soils from more acidic rocks such as yellow-brown pumice soils and the zonal soils. Soil development sequences, representing increasing degrees of weathering and leaching, from groups of parent rocks, show a decrease in the phosphorus content of the topsoils. The greater the iron and aluminium content of the parent rock, the greater is the proportion of the original soil phosphorus retained in the strongly weathered, strongly leached, soils. Soil development is accompanied by a decrease in the solubility in N H_2SO_4 of the inorganic phosphorus which reflects the progressive fixation of the soil phosphorus.

The capacity of topsoils to retain added phosphate has been related to the degree of soil weathering, leaching and podzolisation.

The proportion of phosphorus in organic form is high but does not form a systematic pattern within soil sequences. It is generally low in recent soils and high in organic soils.

The amount of phosphorus taken up by the grass, sweet vernal (*Anthoxanthum odoratum*) from non-topdressed sites is related to the stage of soil development rather than to the amount of phosphorus present. High levels of phosphorus are found in the grass on recent soils and on weakly weathered, weakly leached soils, irrespective of their parent rock, while strongly weathered, strongly leached, soils have low levels of phosphorus in the grass.

Introduction

The New Zealand genetic soil classification (Taylor 1948; Taylor and Cox 1956) presents soils as sequences of development rather than as isolated units. These sequences can be used to show how the processes of soil formation have altered the availability of elements to plants. This survey evaluates the relative levels of plant-available phosphorus using the phosphorus content of the grass, sweet vernal (*Anthoxanthum odoratum*) at the flowering stage (Wells 1956a). Topsoils (A_0 + A_1 horizons) have been analysed for total soil phosphorus, organic phosphorus, inorganic phosphorus, and an inorganic phosphorus soluble in N H_2SO_4.

Methods

Total Soil Phosphorus

A HF + HNO_3 digestion was made on 0.5 g of finely ground topsoil and the excess fluoride was removed by repeated evaporation with HNO_3. The residue was taken up in 25 ml of HNO_3 (132 ml conc. HNO_3/1) and digested for 1 hour on a water bath. The solution was filtered into a 100 ml volumetric flask, and then basin, filter paper, and residue were washed well. The volume was made up to approximately 75 ml, 10 ml of 0.25% ammonium vanadate and 10 ml of 5% ammonium molybdate added (Kitson and Mellon 1944), and then the volume was made up to 100 ml. After 30 min the intensity of the yellow colour was read on a EEL absorptiometer using a blue filter. The results are expressed on an air-dry weight basis.

Organic Phosphorus

The organic phosphorus was calculated as the difference between the inorganic phosphorus extracted from ignited and unignited samples of the soil (Saunders and Williams 1954). This extraction was carried out with N H_2SO_4 for 16 hours at a soil : solvent ratio of 1 : 200.

Inorganic Phosphorus

The difference between the total and the organic phosphorus values gave the inorganic phosphorus. The percentage solubility of the inorganic phosphorus in N H_2SO_4 was calculated from:—

$$\frac{\text{Inorganic P soluble in N } H_2SO_4 \times 100}{\text{Total inorganic P}}$$

Plant Phosphorus

The phosphorus levels of the sweet-vernal plants were determined after ignition at 500°C. The ash was digested with 1 + 1 HCl and baked to dryness to precipitate the silica. The residue was taken up in warm 0.2 N HCl, filtered, and the phosphorus determined by the vanadate-molybdate method. The results were expressed on an oven-dry weight basis.

Phosphate Retention

The capacity of the topsoils to retain phosphate was measured by shaking 1 g of air-dry soil with 25 ml of sodium dihydrogen phosphate solution containing 50 mg P/l. The suspension was filtered and phosphorus determined in an aliquot of the filtrate by the vanadate-molybdate method. The term retention is used to denote removal of phosphate from solution by reaction with the soil.

Results

In Table 1 are the phosphorus levels in sweet vernal from a variety of untopdressed and topdressed sites. The latter sites were last topdressed the autumn before sampling. In all soils except those from recent alluvium there has been a substantial increase in the phosphorus level of the plant with phosphate topdressing. In this survey the mean phosphorus value of the grass from the topdressed sites, 0.26% P, has

been taken as indicating a desirable level of available phosphate for the untopdressed sites given in Tables 2 to 18.

Where possible, soils are considered in sequences, generally representing increasing degrees of weathering and leaching, from chemically similar parent rocks. Where a single soil-forming process dominates the character of the profile, e.g., podzols and organic soils, the soils have been grouped according to the process.

TABLE 1. Effect of Lime + Superphosphate Topdressings on Plant Phosphorus

Soil Group	Genetic No.	Topdressing		Plant P%	
		Lime cwt	Super. cwt	Non-topdressed	After topdressing
Yellow-grey to yellow-brown earth	3b	5	2	.18	.34
Moderately weathered yellow-brown earth	5c	5	3	.07	.17
Podzol	7b	4	2	.11	.25
Yellow-brown pumice	13a	0	3	.15	.31
Yellow-brown loam	14a	0	2	.15	.26
Brown granular clay	15b	3	2	.13	.22
Brown loam	16a	3	3	.12	.31
Brown loam (ironstone)	16c	4	3	.09	.22
Organic soil	19	0	1	.18	.24
Recent alluvium	20b	0	1	.25	.26
Average				.14	.26

Soils from Greywacke

The soils formed from greywacke (Table 2) cover a wide range of climate and vegetation. In the weakly to moderately weathered soils (Omarama, Haldon, Hurunui, Kaikoura, and Tautuku) the total phosphorus values are high and of a similar order to those for greywacke rocks (0.44 to 0.115% P; Reed 1957). In the moderately and strongly weathered soils the total phosphorus values of the topsoils are lower and reach their lowest value in the strongly weathered, strongly podzolised, Hukerenui soil (0.016% P). The proportion of the total phosphorus in organic form is moderately high with no consistent trends within the sequence.

The solubility in N H_2SO_4 of the inorganic phosphorus is very high in the weakly weathered soils of the South Island. It decreases with increased degree of weathering and reaches a minimum in the strongly weathered and strongly leached soils of North Auckland. Within each weathering zone this solubility tends to decrease with increasing degree of soil leaching, apart from the moderately podzolised Hukerenui soil where the small residue is very soluble.

The plant analyses will be considered in three soil groupings: azonal steepland soils, zonal (non-podzolised soils) and podzolised soils.

Plants on the azonal steepland soils (genetic No. 21) have phosphorus values which decrease with increasing intensity of soil weathering. High values occur in the plants on weakly to moderately weathering soils (Haldon and Hurunui), a moderate value is found in the plants on a moderately weathering soil (Makara) and a very low value occurs on a strongly weathering soil (Te Ranga).

In the same weathering zones, plants on the zonal soils generally have lower phosphorus values than those growing on the related azonal steepland soils. The plants on the zonal soils under weak weathering (Omarama and Kaikoura) have slightly higher levels of phosphorus than plants on the soils under moderate weathering (Korokoro and Taita) and under strong weathering (Marua and Rangiora).

Plants on the podzolised soils (Tautuku, Renata, and Hukerenui) have phosphorus values that also decrease with increasing degree of soil weathering. In the weak to moderate weathering zones the plant phosphorus values are higher than those on the related zonal (non-podzolised) soils. Under strong weathering any increase in phosphorus availability by podzolisation is offset by the low total amount of phosphorus present in the soil.

TABLE 2. SOILS FROM GREYWACKE

Sample No.	Weathering Zone	Genetic No.	Soil Type	Total P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
358	weak to moderate	21b	Haldon stony silt loam	.20	.068	71	100
357		21c	Hurunui stony loam	.20	.192	50	94
114		1b	Omarama sandy loam	.15	.116	66	100
115		4	Kaikoura loam	.12	.110	42	81
393		7a (weakly podzolised)	Tautuku silt loam	.27	.098	59	50
449	moderate	21d	Makara stony loam	.17	.038	58	63
353		5b	Korokoro silt loam	.10	.052	50	54
396		5c	Taita hill soils, silt loam	.07	.040	43	39
972		5c (weakly podzolised)	Renata loam	.14	.054	56	33
346	strong	21e	Te Ranga buff clay loam	.08	.042	72	33
333		6b	Marua clay loam	.08	.036	53	53
330		6c	Rangiora clay	.10	.030	63	37
345		7b (moderately podzolised)	Hukerenui silt loam, yellow subsoil	.08	.016	63	100

Soils from Mica Schist

Three soils from mica schist on the Old Man Range in Otago (Table 3), ranging from a steepland brown-grey earth to a weakly weathered yellow-brown earth, have total phosphorus values similar to the weakly weathered South Island soils from greywacke. There is a slight decrease in the soil phosphorus values as the rainfall increases, and this is accompanied by a decrease in the solubility of the inorganic phosphorus.

The plant phosphorus values are high on these soils, the highest values occurring under the lowest rainfall.

TABLE 3. SOILS FROM MICA SCHIST

Sample No.	Genetic No.	Rainfall (in.)	Soil Type*	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
111	21a	18	(Omarama)* sandy loam	.32	.148	44	70
112	—	—	—	.20	.136	55	64
113	4	50	(Kaikoura) loamy sand	.20	.118	58	49

* Provisional soil names and supplementary details of samples are shown in parentheses in all tables.

Soils from Loess

Weakly to moderately weathered soils from loess, mainly yellow-grey earths, in Canterbury, are arranged in Table 4 in order of increasing rainfall. There is a tendency for a decrease in the total soil phosphorus in the sequence except for the Opuha soil which, being close to the source of loess, may have had recent accessions. There is no consistent trend of solubility of the inorganic phosphorus or of the plant phosphorus values, but both values are generally high.

TABLE 4. SOILS FROM LOESS DEPOSITS

Sample No.	Genetic No.	Rainfall (in.)	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
118	1b	18	(Omarama) sandy loam	.25	.140	46	81
220	2c	22	Ngapara fine sandy loam	.26	.090	58	74
326	2c	22	Timaru silt loam	.18	.074	66	60
322	2c	27	Claremont silt loam	.22	.058	67	58
321	2c	33	Opuha silt loam	.25	.090	58	100
73	3b	45	Pawson silt loam	.18	.058	45	88

Soils from Pumiceous Sandstone and Mudstone

Transitions from yellow-grey earths to moderately weathered yellow-brown earths on the pumiceous sandstones and mudstones in Hawke's Bay are given in Table 5. As rainfall increases the total phosphorus values of the topsoil decreases. Under comparable rainfalls the values are similar for sandstone and mudstone. The proportion as organic phosphorus is moderately high. In the topsoils from sandstone the solubility of the inorganic phosphorus in N H_2SO_4 is lower in the soils under higher rainfall but there is little difference between the two soils from mudstone.

TABLE 5. SOILS FROM PUMICEOUS SANDSTONES AND MUDSTONES

Sample No.	Genetic No.	Rainfall (in.)	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P.
	Sandstone						
287	2b	30	Matapiro sandy loam	.26	.038	66	45
277	2b	35	Matapiro sandy loam	.18	.084	58	46
278	3b	40	Rissington sandy loam	.12	.052	65	11
281	5b	50	Matamau silt loam	.09	.042	48	27
	Mudstone						
274	2b	35	Raukawa silt loam	.14	.080	71	57
275	5b	50	(Atua) silt loam	.10	.044	73	67

The plant phosphorus values decrease with increasing rainfall.

Soils from Sandstone

The strongly weathered yellow-brown earths from massive and calcareous sandstones in North Auckland are arranged in Table 6 in order of increasing degree of soil leaching. In these strongly weathered soils the degree of leaching is influenced by factors such as slope and vegetation rather than rainfall. The total phosphorus values are all low and comparable with those of the strongly weathered soils from greywacke (Table 2). They decrease with increasing soil leaching, reaching the lowest level of New Zealand topsoils in the strongly podzolised Wharekohe soil (0.014% P).

As with the strongly weathered soils from greywacke, the solubility of the inorganic phosphorus is lowest at the strongly leached but not markedly podzolised stage (no greyish A_2).

Except for the youngest soil (Atuanui), the plant phosphorus values are all low to very low, and tend to be lower on the soils from massive sandstone than from calcareous sandstone.

Podzolised Soils

The podzolised yellow-brown earths have been listed in Table 7 according to the zone of weathering. In the strongly weathered group

TABLE 6. SOILS FROM SANDSTONE

Sample No.	Genetic No.	Soil Type: Massive Sandstone	Soil Type: Calcareous Sandstone	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
190	21e		Atuanui clay loam	.17	.040	33	70
490	21e	White Cone sandy clay loam		.10	.026	58	45
191	6a		Puhoi hill soils	.12	.040	62	47
148	6b	Waiotira hill soils		.09	.038	53	45
183	6b		Whangaripo clay	.12	.044	27	16
144	7b (mod. podzolised)	Waikare sandy clay		.08	.022	40	33
323	7b (strongly podzolised)	Wharekohe sandy loam		.09	.014	43	50

TABLE 7. PODZOLISED SOILS

Sample No.	Parent Rock	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
Weak weathering zone, genetic No. 7a						
393	Greywacke	Tautuku silt loam	.27	.098	59	50
337	——	Tisbury complex	.19	.056	50	25
Moderate weathering zone, genetic No. 5c						
979	Gravels	Hope silt loam	.13	.090	29	11
972	Greywacke	Renata loam	.14	.054	56	33
Strong weathering zone weakly podzolised, genetic No. 6c						
343	Claystone	Okaka silty clay	.15	.034	41	10
330	Greywacke	Rangiora clay	.10	.030	63	37
moderately podzolised, genetic No. 7b						
144	Sandstone	Waikare sandy clay	.08	.022	40	33
153	Aeolian sand	Tangitiki sand	.11	.014	50	43
moderately to strongly podzolised, genetic No. 7b						
345	Greywacke	Hukerenui silt loam	.08	.016	63	100
323	Sandstone	Wharekohe sandy loam	.09	.014	43	50
89	Alluvium	Kara strongly gleyed silt loam	.14	.026	39	37
103	Aeolian sand	Te Kopuru sand	.07	.018	61	43

they have been subdivided according to the degree of podzolisation. They include samples given in previous tables.

In the weakly and moderately weathered topsoils the total phosphorus values are similar to those of the related non-podzolised soils of the same region. In the strongly weathered topsoils the total phosphorus values are much lower and decrease with increasing degree of podzolisation, reaching their minimum in soils podzolised by predominantly kauri (*Agathis australis*) vegetation.

The proportion of phosphorus in the organic form is high and the small amount of inorganic phosphorus remaining in the strongly podzolised soils can be readily dissolved in N H_2SO_4.

The phosphorus levels in the plants are high on the two weakly weathered soils and low on the moderately and strongly weathered soils.

Soils from Limestone

Topsoils of rendzinas, derived from calcareous sedimentary rocks (Table 8) have slightly higher total phosphorus values than the related yellow-grey and yellow-brown earths given in Tables 2 to 6. The solubility of the inorganic phosphorus is high and approximately double that of the related zonal soils in Tables 2 to 6. The proportion of the soil phosphorus as organic phosphorus is high (mean 63%).

The plant phosphorus values are generally moderate with higher values on the steeper hill soils (Motatau and Konoti). The values are usually greater than those on the related yellow-grey and yellow-brown earths from less calcareous rocks.

TABLE 8. Soils from Limestone (Genetic No. 11)

Sample No.	Weathering Zone	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
223	Moderate	Waikakahi complex (silt loam)	.15	.090	60	75
473	„	Te Mara silt loam	.14	.110	42	78
221	„	Waiareka (clay loam)	.17	.116	51	79
286	„	Te Onepu clay loam	.15	.150	77	74
99	Strong	Motatau hill soils	.20	.068	75	59
110	„	Konoti hill soils	.18	.072	75	56
82	„	Matakohe complex (clay)	.11	.048	69	73
105	„	Dairy Flat complex (clay)	.10	.030	53	64

Soils from Aeolian Sands

The two sequences on yellow-brown sands (Table 9) are a drainage sequence towards peat under moderate weathering conditions in the Manawatu, and a leaching sequence towards a strongly podzolised soil in North Auckland.

In the first sequence, total soil phosphorus values decrease as the drainage becomes poorer until the peat soil, when the value increases owing to a large increase in the organic phosphorus. The solubility of

the inorganic phosphorus is high throughout the sequence and increases as the drainage becomes poorer. Plant phosphorus values tend to decrease with the poorer drainage reaching a very low level on the peaty soil.

In the second sequence increased soil leaching is accompanied by a decrease in total soil phosphorus. The values of all but the youngest soil (Whananaki) are low and comparable with those of the strongly weathered yellow-brown earths. The Whananaki sand has the lowest proportion of organic phosphorus (28%), probably owing to its immaturity. The solubility of the inorganic phosphorus in N H_2SO_4 is moderately high in the Whananaki sand, falls to a low value for the middle members of the sequence and then rises in the strongly podzolised soils.

The plant phosphorus values fall from a moderate value in the Whananaki sand to a very low value in the strongly podzolised Te Kopuru sand.

TABLE 9. SOILS FROM AEOLIAN SAND

Sample No.	Genetic No.	Weathering Zone	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
317	12	Moderate	Foxton black sand	.14	.154	49	60
60	12	„	Himatangi weakly mottled sand	.18	.064	47	68
194	12	„	Pukepuke black sand	.09	.052	64	100
369	19	„	Omanuka peaty sandy loam	.08	.110	74	100
106	12	Strong	Whananaki sand	.19	.138	28	67
85	12	„	Pinaki sand	.12	.030	47	25
155	12	„	Red Hill sandy clay loam	.11	.036	44	35
153	7b (mod. podzolised)	„	Tangitiki sand	.11	.014	50	43
103	7b (v. strongly podzolised)	„	Te Kopuru sand	.07	.018	61	43

Soils from Pumice

The yellow-brown pumice soils occur on the central plateau of the North Island and, because of their recent origin, are only weakly weathered. The total soil phosphorus values in Table 10 reflect the low phosphorus contents of rhyolite (0.055% P; Goldschmidt 1954) and they are lower than those of the weakly weathered zonal soils. The yellow-brown pumice soils are arranged in increasing degree of leaching within each ash shower. It appears that soil leaching may have caused losses of phosphorus only on the older Taupo and Gisborne showers. The separate identity of the Gisborne shower is in some doubt at present. The proportion of the soil phosphorus as organic phosphorus is moderately high (mean 58%). The solubility of the inorganic

TABLE 10. Soils from Pumice

Sample No.	Genetic No.	Ash Shower	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
178	13a	Kaharoa	(Galatea sand)	.13	.042	57	33
179	13a	,,	Oropi sand	.12	.040	50	40
177	13b	,,	Kaharoa sand	.13	.052	42	67
160	13a	Taupo	Taupo (deep sand)	.15	.074	55	45
157	13a	,,	Taupo (brown sand)	.14	.058	48	20
159	13b	,,	Mamaku sandy silt	.15	.048	79	80
304	13a	Gisborne	Gisborne sandy loam	.15	.078	59	56
307	13a	,,	Matawai sandy loam	.10	.048	60	47

phosphorus in these weakly weathered pumice soils is generally lower than in the weakly weathered zonal soils (Tables 2 to 4).

The plant phosphorus values are low and, like the acid solubility, show no trends between or within the ash showers.

Soils from Andesitic Ash

The recent soils (20b), yellow-brown loams (14), and a brown granular clay loam (15) from andesitic ash have been arranged (Table 11) in order of increasing age of the parent ash shower and within each shower according to the degree of leaching. The total phosphorus value in most of these soils is higher than those of the yellow-brown earths or the yellow-brown pumice soils. Goldschmidt (1954) quotes andesites as having a mean phosphorus content of 0.125%. The soils from the Egmont volcano (Burrell, Stratford and Egmont showers) have the highest phosphorus values. Within this group of soils the total phosphorus values increase with age, probably owing to a small change in the parent rock. Within the Mairoa shower the soil phosphorus values decrease with increasing leaching. The oldest and most weathered soil, a brown granular clay loam from the Hamilton shower, has the lowest phosphorus value.

The proportion of the soil phosphorus as organic phosphorus is generally high, it averages 60% for the yellow-brown loams. These high values have been related by Jackman (1955) to the stabilisation of organic matter by allophane, the dominant amorphous clay mineral of these soils (Taylor 1933; Fieldes 1955). The acid solubility of the inorganic phosphorus ranges from low in the Ohakune soil to high in the Egmont and Mairoa soils. Within the Mairoa shower there is a decrease of solubility with increased leaching. The solubility in the Hamilton shower is low.

The plant phosphorus values on the younger yellow-brown loams are only moderate in spite of their high total soil phosphorus and the

TABLE 11. SOILS FROM ANDESITIC ASH

Sample No.	Genetic No.	Ash Shower	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
164	20b	Burrell	Burrell gravelly sand	.13	.154	40	55
241	20b	Ngauruhoe	Waimarino sand	.12	.082	33	47
165	14d	Stratford	Inglewood coarse sandy loam	.13	.188	62	61
163	14d	Stratford	Patua sandy loam	.16	.182	65	65
301	14a	Tongariro	Ohakune silt loam	.16	.096	65	29
168	14a	Egmont	Egmont black loam	.15	.266	47	83
167	14a	Egmont	New Plymouth black loam	.16	.226	59	91
247	14a	Egmont	New Plymouth brown loam	.16	.254	49	94
172	14a	Mairoa	Ohaupo silt loam	.12	.126	72	89
303	14a	Mairoa	Te Kuiti silt loam	.11	.102	54	74
836	14d	Mairoa	Mairoa silt loam	.12	082	70	60
65	15a	Hamilton	Hamilton clay loam	.10	.040	65	43

generally high solubility of the inorganic phosphorus. In the older, more leached, showers (Mairoa and Hamilton), the plant phosphorus levels are low.

Brown Granular Clays from Andesite

The brown granular clays (Table 12) occur on andesite in North Auckland and they represent a stage of soil development beyond the yellow-brown loams from andesitic ash given in the previous table. Increasing soil leaching is accompanied by a decrease of the total phosphorus value of the soils. The proportion in the organic form (average 43%) is lower than in the yellow-brown loams. The solubility of the inorganic phosphorus is low throughout the sequence and the plant phosphorus values are also low.

Soils from Basalt

The recent soils (20b) and red-brown loams (16), mainly from olivine basalts, in North Auckland cover a wide range of soil develop-

TABLE 12. SOILS FROM ANDESITE (Genetic No. 15)

Sample No.	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
133	Awapuku clay loam	.10	.080	46	21
132	Awarua clay	.13	.050	40	40
474	Rangiuru clay	.13	.048	50	25
477	Rangiuru clay (strongly gleyed)	.10	.032	34	33

ment (Table 13). Recent eruptions have given weakly weathered and weakly leached soils, while on the older flows the soils are strongly weathered and strongly leached. The total phosphorus values of the weakly weathered soils are similar to the phosphorus content of basalts (0.244% P; Goldschmidt 1954). The high level of phosphorus in South Island brown loams has been noted by Walker and Adams (1958). With increasing soil weathering and leaching the total phosphorus values decrease to 0.044% P in an Okaihau ironstone soil. The proportion of the soil phosphorus in organic form (average 43%) is low compared with that of most other soil groupings. Accompanying the decrease in the total phosphorus value of these soils is a change in the solubility of the inorganic phosphorus from very high values in the weakly weathered soils to very low values in the strongly weathered, ironstone, soils. At this final stage of soil weathering from basalt, the phosphorus in the organic matter is very low.

The plant phosphorus values are high on the recent soils (20b) and moderate to low on the weakly or moderately leached (16a) soils. A loss of phosphorus, accompanied by marked decrease in solubility of the residue, results in very low phosphorus values in plants on the strongly weathered soils. The Okaihau soil, near Kerikeri, has the lowest levels of phosphorus in sweet vernal so far found on a mineral soil in New Zealand.

TABLE 13. Soils from Basalt

Sample No.	Genetic No.	Nature of Basalt	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
882	20b	Ash	Rangitoto sandy loam	.24	.202	40	100
835	20b/16a	Scoria	Papakauri stony loam	.21	.202	53	77
123	16a	Flow, ash	Ohaeawai bouldery silt loam	.16	.336	42	85
101	16a	Flow, ash	Ohaeawai silt loam	.12	.228	57	86
834	16a	Flow, ash	(Kiripaka) bouldery silt loam	.17	.358	29	90
151	16a	Flow	Kiripaka bouldery silt loam	.12	.110	47	41
93	16a	Scoria	Maunu silt loam	.18	.138	59	35
76	16b	Scoria	Apotu friable clay	.17	.168	54	34
81	16b	Flow	Waiotu friable clay	.11	.100	42	10
92	16b	Flow	Kerikeri friable clay	.09	.160	43	20
94	16c	Flow	Taraire gravelly friable clay	.08	.106	25	8
87	16c	Flow	Okaihau gravelly friable clay	.09	.078	45	7
377	16c	Flow	Okaihau gravelly friable clay	.06	.044	23	6

Gley Soils

Soils formed on gleyed alluvium derived from various parent rocks are listed in Table 14. The two from basaltic and andesitic alluvium in North Auckland are strongly leached clays and, like their related non-gleyed soils (Tables 13 and 14), they have low total phosphorus values. Their inorganic phosphorus solubilities are likewise low. The gley soil from pumice alluvium is weakly weathered, its total phosphorus, and the solubility of the inorganic fraction, is higher than that of the yellow-brown pumice soils.

The levels of phosphorus in the plant are variable and depend upon the nature of the alluvium rather than the influence of gleying. Plants on gleyed alluvium have phosphorus levels that are akin to those on the related non-gleyed alluvium. For example, the phosphorus value of plants on the Waipapa soil are very low and are similar to those on the Okaihau ironstone soils (Table 13). Plants on the Motukarara soil have high values like those on the Waimakariri soil (Table 16).

TABLE 14. GLEY SOILS (Genetic No. 17 and 18)

Sample No.	Alluvium from	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
351	Basalt	Waipapa clay	.07	.042	50	19
468	Andesite	Pakotai clay	.15	.048	38	20
243	Pumice	Waitoa silt loam	.12	.136	52	88
327	Sed. + ign.	Makarewa silt loam	.13	.088	68	68
462	Sedimentary	Motukarara sand loam (saline)	.20	.058	29	81
79	Sedimentary	Kaipara clay	.20	.070	47	35
78	Sedimentary	Waipu clay	.10	.082	51	30

Organic Soils

The total phosphorus values of the organic soils (Table 15) are variable. In this group the proportion of the soil phosphorus in organic form (average 70%) is generally higher than in the mineral soils. There is no apparent trend in the solubility of the inorganic phosphorus. Alluvial mineral matter in the Waimairi site amounts to 50% and makes a considerable contribution to the total phosphorus.

The plant phosphorus values are low to very low and tend to be related to the total soil phosphorus.

Recent Soils from Alluvium

The recent soils formed on alluvium (Table 16) have total phosphorus values that are related to those of similarly weathered soils formed *in situ* over the parent rocks. The soil from andesite has higher phosphorus values than those from rhyolite or the sedimentary rocks. The proportion of the soil phosphorus in the organic form is high, except in the very recent Waimakariri and Manawatu soils. The pro-

TABLE 15. ORGANIC SOILS (Genetic No. 19)

Sample No.	Underlying Sediments	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
469	Andesitic alluvium	Pakotai peaty loam	.10	.062	66	33
848	Sed. rock alluvium	Mangaroa peat	.11	.060	85	100
328	Sed. rock alluvium	Invercargill peaty loam	.13	.120	58	73
445	Sed. rock alluvium	Waimairi peaty loam	.18	.140	60	54
329	Aeolian sand	Ruakaka loamy peat	.06	.028	46	33
336	Aeolian sand	Paraparaumu peaty loam	.06	.032	69	40
369	Aeolian sand	Omanuka peaty sandy loam	.08	.110	74	100

portion of the inorganic phosphorus soluble in N H_2SO_4 is related to the nature of the parent rock. The solubility of the soil inorganic phosphorus tends to be higher when the alluvium is from sedimentary rather than from igneous rocks.

The phosphorus values in the plant are high except on the Kaiti (cf. yellow-brown pumice soils, Table 10) and the Whakapara (cf. strongly weathered yellow-brown earths, Table 2).

TABLE 16. RECENT SOILS FROM ALLUVIUM (Genetic No. 20a)

Sample No.	Alluvium from	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
495	Andesite	Mangakahia clay	.19	.122	63	47
72	Rhyolite + sed. rocks	Kaiti silt loam	.12	.062	71	39
383	Greywacke	Waimakariri sandy loam	.25	.062	32	79
382	Sedimentary rocks	Manawatu sandy loam	.24	.064	41	95
245	Sedimentary rocks	Kairanga silt loam	.25	.072	71	48
492	Sedimentary rocks	Whakapara clay loam	.13	.058	74	100

Recent Soils from Volcanic Ash

As the recent soils from volcanic ash have undergone the least weathering of primary minerals the total phosphorus values (Table 17) are close to those of the parent rocks. Recent soils from basalt and andesite have high phosphorus values. The proportion of the soil

phosphorus as organic phosphorus is generally low, the lowest value being found in the Rotomahana soil (errupted 1886).

The plant phosphorus values of the recent soils from basalt, from volcanic lapilli and mud are high. The lower values on the two soils from andesite are probably due to strong surface leaching under high rainfalls. The plant phosphorus values are directly related to the acid solubility of the inorganic phosphorus.

TABLE 17. RECENT SOILS FROM VOLCANIC ASH (Genetic No. 20b)

Sample No.	Parent Rock	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
882	Basaltic ash	Rangitoto sandy loam	.24	.202	40	100
835	Basaltic scoria	Papakauri stony loam	.21	.202	53	77
164	Andesitic ash	Burrell gravelly sand	.13	.188	40	55
241	Andesitic ash	Waimarino sand	.12	.082	33	47
229	Volcanic lapilli	Tarawera gravel	.21	.050	—	—
239	Rhyolitic mud	Rotomahana sandy loam	.21	.054	13	60

Steepland Soils

Steepland soils related to the zonal soils are arranged in Table 18 according to their weathering zone. The total phosphorus values are markedly higher in the weakly weathering South Island skeletal soils

TABLE 18. STEEPLAND SOILS RELATED TO ZONAL SOILS (Genetic No. 21)

Sample No.	Genetic No.	Parent Rock	Soil Type	Plant P g/100 g	Total Soil P g/100 g	Org. P × 100 / Total P	Acid-sol. P × 100 / Inorg. P
111	21a	Mica schist	(Omarama) sandy loam	.32	.148	44	70
358	21b	Greywacke	Haldon stony silt loam	.20	.068	66	100
357	21c	Greywacke	Hurunui stony loam	.20	.192	71	100
112	21c	Mica schist	(Hurunui) loam	.20	.136	55	64
405	21d	Moutere gravels	Spooners stony silt loam	.14	.088	45	19
449	21d	Greywacke	Makara stony loam	.17	.038	58	63
256	21d	Mudstone	Taihape silt loam	.18	.050	68	87
346	21e	Greywacke	Te Ranga buff clay loam	.08	.042	72	33
190	21e	Calc. sandstone	Atuanui clay loam	.17	.040	33	70
490	21e	Sandstone	White Cone sandy clay loam	.10	.026	58	45

(genetic Nos. 21a, b, c, d) than in the moderately and strongly weathering North Island skeletal soils (genetic Nos. 21d, e). Many of these steepland soils have over 60% of the soil phosphorus in organic form. The acid solubility of the inorganic phosphorus is high except for the strongly weathered Te Ranga and White Cone soils in North Auckland, and the Spooners soil from the Moutere Gravels in Nelson. Similarly, the plant phosphorus levels are moderate to high in all but these three soils.

Phosphate Retention

The percentage of added phosphate retained by topsoils from two groups of parent rocks are given in Tables 19 and 20. The zonal soils, derived from siliceous rocks, have been grouped into weathering zones and subdivided according to degree of leaching and podzolisation. Within each weathering zone the phosphate retentions show a maximum at the most leached stage before podzolisation. This maximum phosphate retention increases from the weak to the strong weathering zone and may be attributed to the accumulation of secondary iron and aluminium minerals. In terms of the secondary iron released during soil weathering (Aguilera and Jackson 1953) the difference between a weakly weathered soil (Omarama) and a strongly weathered soil (Marua) was 0.9 and 3.0% Fe. The secondary iron values have been taken from a survey by Dr G. Claridge (in preparation). Within each weathering zone the maximum phosphate retention is the resultant of two factors, pH and podzolisation. If leaching is accompanied by podzolisation, the effect of a lower pH on the retention value is nullified by a loss of iron and aluminium from the topsoil. A decrease in pH leads to a fall in the concentration of hydroxyl ions that are available to saturate the bonds that could retain anions such as phosphate, while podzolisation removes the actual sites of bonding. The retention of phosphate ions is considered to be analogous to the retention of molybdate ions (Wells 1956b). The magnitude of these effects, hydroxyl concentration and podzolisation, are greatest in the strongly weathered soils.

The more recent basaltic eruptions, in the zone of strong weathering, have given weakly weathered soils that are only weakly leached (Table 20), but owing to their high levels of iron and aluminium they have high retentive capacities for phosphate. The fast weathering of the basalt has resulted in a secondary iron level of 2.5% Fe in the youngest soil (Rangitoto). The retentions of these young soils are of a similar order to those of the moderately weathered, moderately leached, zonal soils (secondary iron on Korokoro soil 1.3% Fe). Although the amount of secondary iron minerals increases as the soils become more weathered, culminating in an ironstone soil with 7.5% secondary Fe, the phosphate retentions reach a maximum at an earlier stage when the soils are only moderately weathered (silt loams). This climax in the soil sequence is probably related to a build-up of the hydrous oxides of iron and aluminium, followed by a change from amorphous to mainly crystalline forms (Fieldes and Swindale 1954) with a consequent decrease in colloidal surface area.

TABLE 19. PHOSPHATE RETENTION BY ZONAL TOPSOILS

Weathering Zone	Leaching	Genetic No.	Parent Rock	Soil Type	% Phosphate Retention	Soln. pH
Weak	Weak	1a	Mica schist	Alexandra sdy. l.	0	6.4
	Moderate	1b	Greywacke	Omarama sdy. l.	0	5.6
	Moderate	4	Greywacke	Kaikoura l.	15	5.5
	Moderate (podzolised)	7a	Basic greywacke	Tautuku silt l.	0	5.0
Moderate	Weak	2b	Calc. mudstone	Raukawa silt l.	6	5.7
	Weak	2b	Sandstone	Matapiro sdy. l.	20	5.3
	Moderate	3b	Greywacke	Pawson silt l.	20	5.0
	Moderate	2c	Greywacke	Claremont silt l.	22	5.2
	Moderate (strongly gleyed)	2e	Mica schist	Warepa silt l.	16	5.8
	Weak	5a	Mudstone	Atua silt l.	26	5.5
	Moderate	5b	Greywacke	Korokoro silt l.	44	5.3
	Strong	5c	Greywacke	Taita silt l.	54	4.6
	Strong (weakly podzolised)	5c	Greywacke	Renata l.	0	4.2
Strong	Weak	6a	Calc. sandstone	Puhoi c. l.	3	5.4
	Moderate	6b	Greywacke	Marua c. l.	63	5.2
	Strong	6c	Greywacke	Rangiora c. l.	60	5.2
	Strong (Weakly podzolised)	6c	Greywacke	Rangiora silty c. l.	40	5.0
	Strong (moderately podzolised)	7b	Greywacke	Hukerenui silt l.	38	4.8
	Strong (strongly podzolised)	7b	Sandstone	Wharekohe sdy. l.	0	4.8

TABLE 20. PHOSPHATE RETENTION BY TOPSOILS FROM BASALT

Weathering Zone	Leaching	Genetic No.	Soil Type	% Phosphate Retention	Soln. pH
Strong	Weak	20b	Rangitoto sdy. l.	41	6.8
	Weak	20b/16a	Papakauri stony l.	48	6.0
	Weak	16a	Ohaeawai silt l.	91	5.3
	Moderate	16a	Kiripaka b. silt l.	63	5.6
	Moderate	16a	Whakapai c. l.	65	5.5
	Strong	16b	Waiotu f. c.	43	5.3
	Strong	16b	Kerikeri f. c.	38	5.4
	Strong (ironstone)	16c	Taraire g. f. c.	38	5.9
	Strong (ironstone)	16c	Okaihau g. f. c.	56	5.9

Discussion

The total phosphorus values for the topsoils of weakly weathered soils are directly related to the values associated with the rocks from which they have been formed.

	% P Rock	Weakly weathered topsoil	Strongly weathered topsoil
Basalt	0.24 (1)	0.24	0.11
Andesite	0.12 (1)	0.15	0.05
Rhyolite	0.06 (1)	0.05	—
Greywacke	0.10 (2)	0.12	0.015

(1) Goldschmidt (1954) (2) Reed (1957)

With increasing degree of weathering and leaching the concentration of phosphorus in the topsoils decreases. A similar trend was found by Walker and Adams (1959) in their study of a sequence of soil profiles formed from greywacke. The ratio of total soil phosphorus in weakly weathered soils to that in the strongly weathered soils differs with nature of parent rock. At corresponding degrees of weathering the soils from basalt have held a greater proportion of phosphorus than those from andesite, which, in turn, have held more than those from greywacke. The proportion of the phosphorus held in the strongly weathered soils is directly related to the amount of secondary minerals of iron and aluminium.

The decreasing total soil phosphorus values within soil sequences are accompanied by decreasing levels of available phosphorus as indicated by the phosphorus content of the sweet-vernal plants. However, although the decreasing concentration of phosphorus in the soil contributes to the fall in available phosphorus, the major cause is more probably the change in the nature of the inorganic phosphorus. This change is reflected in its decreasing solubility in N H_2SO_4 and may be regarded as resulting from the progressive fixation of the original soil phosphorus under the influence of soil weathering, leaching and secondary mineral formation. The high solubility of inorganic phosphorus in the weakly weathered soils suggests that the phosphorus is present mainly as Ca-bound phosphorus, forms of apatite, and as phosphate held on the surface of colloidal hydrous oxides of iron and aluminium. With increasing degree of weathering and leaching it may be supposed that the calcium-bound phosphorus is converted to iron- and aluminium-bound phosphorus. Moderately weathered soils, especially those from volcanic ash (yellow-brown loams, genetic No. 14, Table 11) illustrate this stage in the fixation of inorganic phosphorus. The level of available phosphorus is low but the solubility of the inorganic phosphorus in N H_2SO_4 is still high, indicating that this phosphorus is mainly at or near the surface of the soil inorganic colloids. A final stage in the fixation of the inorganic phosphorus is shown by strongly weathered soils, particularly the gibbsitic ironstone soils from

basalt (Table 13). In these soils both the available phosphorus and the solubility of the inorganic phosphorus in N H_2SO_4 are very low. This has probably been brought about by the phosphorus bound at iron and aluminium surfaces becoming occluded (Chang and Jackson 1957) by subsequent deposition of iron and aluminium hydrous oxides and, at the same time, there may have been a change towards crystallinity in the inorganic phosphorus.

Besides fixation by the mineral fraction of the soil, a considerable proportion of the soil phosphorus is fixed by the organic fraction. Most of the soils which gave low and very low levels of phosphorus in sweet vernal have sufficient organic phosphorus to give a high level of phosphorus to plants if it were readily available. It can only be concluded that, at least in the more weathered and leached soils, the organic phosphorus fraction plays little or no part in the immediate nutrition of plants.

The level of available phosphorus, as indicated by the phosphorus content of the flowering sweet vernal, relates to the stage of soil development rather than to the total phosphorus content of the soil. Recent soils from alluvium or volcanic ash, and weakly weathered, weakly leached soils, irrespective of their parent rock, gave phosphorus values in the sweet vernal that are only slightly lower than the average value attained from sites that had been topdressed with phosphate.

Low levels of available phosphorus occur in the strongly weathered, strongly leached, soils, irrespective of the total soil phosphorus. These low values may be the result of one or more of three soil processes; decrease in the concentration of phosphorus in the topsoil, fixation of phosphorus by secondary minerals, and fixation by organic matter. Examples can be given where one process is dominant. The Wharekohe sandy loam, a strongly weathered, strongly podzolised soil (Table 6), has only 0.014% P in the highly siliceous topsoil and grows plants with 0.09% P (about a third of the desirable level). The Okaihau gravelly friable clay, a strongly weathered strongly leached, gibbsitic ironstone topsoil (Table 13) has 0.044% P and grows plants with only 0.06% P (about a quarter of the desirable level). In the first soil the concentration of phosphorus in the siliceous topsoil is extremely low and although the small residue of inorganic phosphorus is not all firmly fixed (solubility in N H_2SO_4 50%), it is apparently at too low a concentration to give a high level of phosphorus to the plants. In the second soil, high in iron and aluminium, there is a reasonable amount of phosphorus present but it has been very firmly fixed in the inorganic form (solubility in N H_2SO_4 6%). The generally low phosphorus levels in the sweet vernal on the organic soils (Table 15), especially those with little mineral accession, provide the third example illustrating the low availability to plants of the organic phosphorus.

CONCLUSIONS

Analyses of a grass, sweet vernal, sampled in relation to sequences of soil development, show the degree to which phosphorus in the topsoil can be rendered unavailable to plants. Decrease of phosphorus

concentration in topsoils and fixation of the remaining phosphorus by mineral matter and by organic matter provides a background to the phosphate fertiliser requirement of New Zealand grasslands. It was not within the scope of this paper to state the actual amounts of phosphate needed by a particular soil as this, at present, can best be ascertained from field trials. However, combination of the phosphorus level in a plant and the evaluation of phosphate retention of the soil indicates the relative amounts of phosphate fertiliser required. These two values are summarised in the appendix according to the groupings of the genetic soil classification.

Acknowledgments

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APPENDIX

PHOSPHORUS LEVELS IN SWEET VERNAL AND PHOSPHATE RETENTION OF TOPSOILS

Genetic Soil Classification (Taylor 1948; Taylor and Cox 1956)	Genetic No.	Plant P	Phosphate Retention
Zonal			
Brown-grey earths:			
weakly leached	1a	—	Low
moderately leached	1b	High to moderate	Low
Yellow-grey earths:			
weakly leached	2b	High	Low to moderate
moderately leached	2c	High to moderate	Moderate
strongly gleyed	2e	Moderate	Moderate
Yellow-brown earths:			
moderately weathered, weakly leached	5a	Moderate	Low to moderate
moderately leached	5b	Moderate to low	Moderate to high
strongly leached	5c	Low	High
Yellow-brown earths:			
strongly weathered, weakly leached	6a	Low	Low
moderately leached	6b	Low	High
strongly leached weakly podzolised	6c	Low	High to moderate
strongly leached, strongly podzolised	7b	Very low	Moderate
Intrazonal			
Rendzinas	11	Moderate	Low to moderate
Yellow-brown sands	12	Moderate to low	Low to moderate
Yellow-brown pumice soils	13	Low	Low to moderate
Yellow-brown loams	14	Moderate to low	High
Brown granular clays	15	Low	High
Red-brown loams	16	Moderate to very low	High
Gley soils	17	Variable	Variable
Organic soils	19	Low to very low	Low
Azonal			
Recent soils:			
from alluvium	20a	High to moderate	Low
from volcanic ash	20b	High to moderate	Moderate
Skeletal soils, related to zonal	21	Variable	Low to moderate

SOME OBSERVATIONS ON THE OCCURRENCE OF *Sporidesmium bakeri* Syd. AND FACIAL ECZEMA DISEASE IN THE FIELD

By R. H. Thornton, Soil Bureau, Department of Scientific and Industrial Research, Wellington, and D. P. Sinclair, Animal Research Station, Manutuke, Department of Agriculture

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Summary

The incidence in pastures of the spores of *Sporidesmium bakeri*, observed to grow saprophytically on dead pasture-plant material, has been studied with a simple spore-counting method.

High spore counts were favoured by large amounts of dead and damaged plant materials and warm, moist conditions.

A relationship has been established between relatively high counts of spores and facial eczema disease in lambs.

A correlation has been shown between spore counts and "beaker test" values.

The incidence of spores was higher in pasture samples from Gisborne and Palmerston North than in samples from Hutt Valley, Lincoln and Gore.

Introduction

A microbiological approach to the facial eczema disease problem in sheep led to the testing of a number of soil and leaf litter fungi isolated from pastures associated with outbreaks of the disease (Thornton and Ross 1959). One of these fungi, *Sporidesmium bakeri* Syd. (= *Scheleobrachea echinulata* (Speg.) Hughes) isolated from grass at a toxic period, when fed to experimental animals caused the development of characteristic liver lesions of the disease. (Thornton and Percival 1959; Percival 1959; Mr M. M. MacKinnon, pers. comm.). Although the liver lesions produced in these experimentally fed animals were very similar to those found in animals suffering from the disease in the field (Mr MacKinnon, pers. comm.), there is as yet little direct evidence linking the fungus *S. bakeri* with the disease as it occurs in the field except for a preliminary observation made by the writers (Thornton and Sinclair 1959). This communication provides a detailed account of investigations on the occurrence of spores of the fungus *Sporidesmium bakeri* in the field, and their relationship to facial eczema disease of sheep.

As large numbers of spores had been seen on grass leaves from a toxic area at Palmerston North and on a gangmower used on Claudelands Showgrounds, Hamilton, at a toxic period (Percival and Thornton 1958), and as toxicity was associated with higher sporing strains of *S. bakeri* (Thornton and Ross 1959) it appeared likely that the disease might be caused by spores of the fungus. If so, then outbreaks of the disease in the field might be expected to follow an increase in numbers of spores of *S. bakeri*. A technique for counting spores of

S. bakeri on pasture therefore was sought. It was expected that results by such a technique might also provide information on the factors influencing spore production in the field.

METHODS AND MATERIALS

Spore Counting Technique

Twenty-five grammes of fresh grass, cut at a height of ½ in. with hand box shears, was added to tap water (250 ml plus 1 drop of 'Teepol' detergent) and shaken vigorously by hand for 1 min in a 2 lb Agee preserving jar. The contents were then strained through a 2 mm sieve. Counts of spores of *S. bakeri* were made on the washings with the use of a Neubauer counting chamber. In each chamber the spores in five 1 sq mm grid squares (centre and four corners) were counted. It was found that spores of *S. bakeri* could be distinguished from those of other similar septate and coloured spores of other fungi, e.g., *Alternaria* sp., by the characters described by Hughes (1953). Ten separate grass samples were taken from each one acre field at equal intervals along each diagonal. Two counting chambers were prepared for each sample. The accuracy and reproducibility of this technique were found, on statistical analysis by Mr W. B. Taylor (pers. comm.), to be good.

Throughout this paper "counts" are expressed in units of 100,000 spores per gramme of dry herbage. They must be regarded as minimum values. The percentage recovery of spores is not known.

Herbage Dry Weight

Determination of herbage dry weights was carried out on samples of herbage dried overnight at 110°c.

Beaker Tests

Beaker tests on dried herbage samples from fields at Manutuke Research Station were carried out by Miss J. Kirk using the method described by Sandos, Clare, and White (1959). Bulked pasture samples of at least 20 lb fresh weight were taken by motor mower at random in a field. These were dried in a hot-air drier at 100°c and thoroughly mixed before a sample for beaker test was taken.

Locations

Counts of *S. bakeri* spores were made on pasture samples from several districts from Gisborne to Gore. Observations were concentrated at Gisborne because it was the only district covered in this investigation in which outbreaks of facial eczema had occurred with any degree of regularity in the past.

Observations at Gisborne were conducted on the Manutuke Research Station, a facial eczema research area of the New Zealand Department of Agriculture, situated on the soil types Matawhero silt loam, Kaiti heavy silt loam and Waihirere heavy silt loam, of the Gisborne Plains. Spore counts were made on a number of one acre fields of similar ryegrass and white clover pasture subjected to various treatments planned to influence the growth of *S. bakeri*. These treatments

had been suggested at a combined meeting of workers of the New Zealand Departments of Agriculture and Scientific and Industrial Research engaged on the facial eczema problem. The fields and treatments were—

Field No. 10: Control. 4–6 in. long pasture in January 1959, containing a high proportion of dead ryegrass stalks.

Field Nos. 20 and 31: Closely mown to a height of ½ in., at 7–14 day intervals, according to rate of growth, since the spring flush of pasture in October, 1958. In field 20, mower clippings were left on the pasture, whereas in field 31 a catcher was attached and the mower clippings were removed. Neither field was grazed from October 1958 to January 1959. As a result of mowing, both fields became like lawns and remained green throughout summer and autumn and contained a high proportion of white clover. In field 31 there were more dead ryegrass plants.

Field. No. 37: Harrowed, with short tine and chain grass harrows, at 7 day intervals from 20 January until 12 March, 1959. Initially similar to field No. 10 but later damaged by too frequent harrowing and became shorter and contained a large quantity of dead ryegrass tillers.

Field No. 19: Calcium hydroxide applied at the rate of 10 cwt/acre on 20 January, 1959.

Field No. 35: Superphosphate applied at the rate of 6 cwt/acre on 20 January, 1959.

Field No. 25: Fungicide, dichlone* (2,3-dichloro-1,4-naphthoquinone) applied at rates per acre of 1 lb on 17 January, 2 lb on 28 February, and 4 lb on 16 March, 1959—each in 200 gallons of water.

Field. No. 1–5: Rotationally grazed, and rotationally mown for bulk collection of grass, every two days. These fields were sampled from time to time.

Herbage in all fields was of similar height and composition when treatments were first applied and all fields, except Nos. 20 and 31, had been set stocked with ewes and lambs since the previous lambing in August 1958.

Samples of herbage from Palmerston North came from a pure ryegrass pasture maintained by the Plant Chemistry Division, DSIR (Butler 1958).

Samples of herbage from Lincoln and Gore came from the respective sub-stations of Grasslands Division, DSIR.

Other samples examined were collected during the course of other investigations being carried out by officers of the Soil Bureau.

Observations in the Wellington District were carried out at Trentham Memorial Park and Taita Experimental Station, in the Hutt Valley.

Sheep

The sheep employed in grazing trials at Manutuke were shorn Romney wether lambs obtained from the Waerenga-o-kuri Soil Con-

* Trade name, Phygon XL.

servation Reserve on hill country near Gisborne. This flock was considered to be free from facial eczema at the time because 12 lambs, slaughtered on 8 January showed no evidence of facial eczema liver damage. These lambs were held, until required, on turnips and were judged to be safe from liver damage. This was confirmed in eight lambs which showed no liver damage when slaughtered after being held on turnips during the whole season.

RESULTS AND DISCUSSION

Occurrence of S. bakeri

Initial observations in November 1958 showed that sporing colonies of *S. bakeri* were present on dead grass leaves at Manutuke Research Station and Makaraka Race Course, Gisborne, and at Upper Hutt.

Occurrence of S. bakeri *at Gisborne*

Further observations were made on Manutuke Research Station in early January 1959 following the occurrence in the period 19-24 December 1958 of warm, moist weather conditions generally regarded as being associated with outbreaks of facial eczema, particularly if occurring in the autumn months. Spores of *S. bakeri* were recorded from pasture samples from a number of fields, in particular large numbers of spores were recorded from field No. 20 (mow and return), where the count was about 14. However, when litter from the A_0 soil horizon was examined the count was 102. Direct microscopical examination established that *S. bakeri* was growing and sporing profusely on the dead grass and clover remains. (Figs. 1 and 2.) Subsequent observations have established that this fungus grows saprophytically on dead pasture-plant material. By the use of petri dish culture plates, exposed at various heights from ground level to

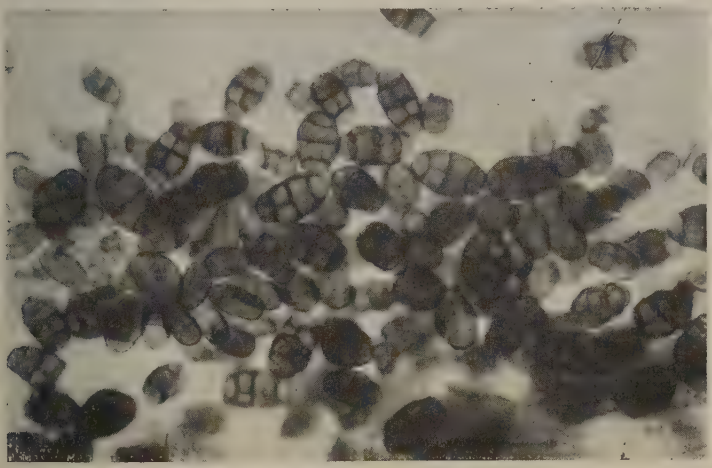

Fig. 1.—Spores of *S. bakeri* on dead grass leaf.

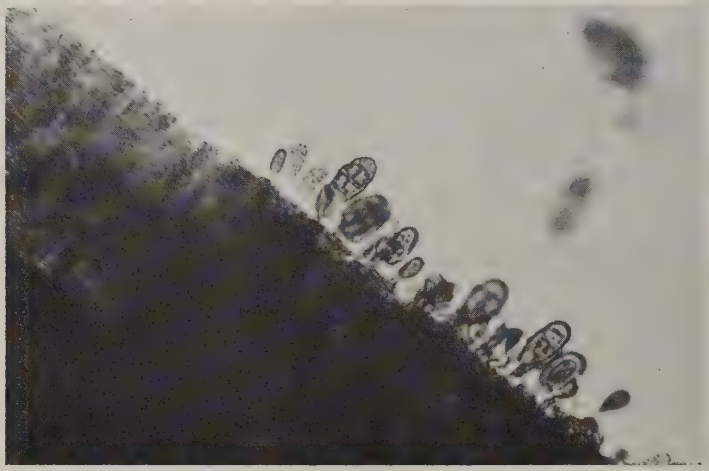

Fig. 2.—*S. bakeri* on dead grass leaf showing stages in the formation of spores.

fifteen feet, spores were found to blow in the wind, particularly when the surface of the ground was disturbed, and microscopical examination revealed spores adhering to living grass and clover leaves. Thus spores on both dead and live material would be ingested by stock.

On dead plant material, spores of *S. bakeri* occurred as small black spots or patches, depending on the amount of spores, but it was not the only fungus producing dark spots and patches.

As a result of these observations four fields at Manutuke Research Station were sampled at approximately weekly intervals. These fields were: No. 10—Control.
No. 20—Mow and return.
No. 31—Mow and remove.
No. 37—Harrowed.

Sampling began of fields 10, 20, and 31 on 9 January, and of field 37 on 18 February 1959, and ceased in all fields on 5 May 1959. Where sampling and mowing of fields 20 and 31 occurred on the same day, sampling was carried out before the pastures were mown.

Spore counts were made on samples from other fields at rather more irregular intervals.

The spore counts obtained on the four fields sampled at approximately weekly intervals are shown in Fig. 3, together with grass minimum temperatures and rainfall.

Direct microscopical examination showed *S. bakeri* colonising and sporulating on recently cut grass and clover, on the cut edges of leaves and on older dead grass.

Results given in Fig. 3 show that the treatments applied to the various fields had a marked effect on spore counts. Not only did the maximum count vary between fields but these maxima occurred at different times. These variations are considered to be due to differences in both the amounts of dead or damaged plant material available to this saprophytic fungus at different times, and the microclimatic conditions favouring fungal growth and sporulation. Thus, in the short mown pasture of field 20 high counts were recorded in January where substantial quantities of herbage were cut on this field in December and January when there were several periods of warm moist conditions favourable to fungus growth and sporulation. The greater additions of freshly killed plant material in field 20 apparently caused higher spore counts and an earlier maximum count compared with field 31. The return of clippings in field 20 would tend to maintain humid conditions within the pasture. After mid January, pasture growth was slow in these two fields until the lambs were removed at the end of

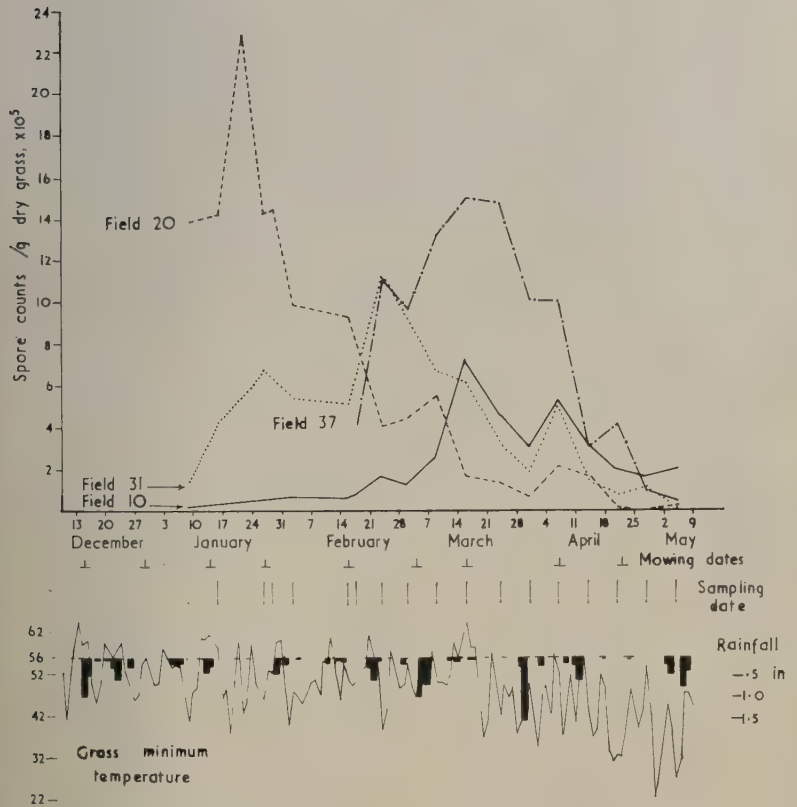

Fig. 3.—Counts of *S. bakeri* spores on pasture in fields at Manutuke Research Station with rainfall and grass minimum temperatures.

March. In the initially longer pastures of fields 10 and 37, maximum counts were recorded in mid March following a warm showery period when fresh pasture growth occurred. More rapid decomposition of old dead plant material occurred with the more humid conditions under the longer pasture. High spore counts were recorded under these conditions. The higher counts in field 37, compared with field 10, are attributed to the greater quantity of dead and damaged plant material produced in that field by regular harrowing.

The rapid decline in counts in some fields could have been produced by a number of factors working singly or together in greater or less degree, e.g., decline in optimum microclimatic conditions; dilution by further pasture growth; decline of available food supply to the fungus either because it had been used up or because of less pasture growth, possibly as a result of injury to plants by the decomposing thick layer of cuttings in field 20; removal of upper portions of plants possibly containing greater concentrations of spores, by grazing sheep or by mowers; rain washing spores away. It is not proposed to attempt to determine which factors were operating in particular fields. Some of these factors may have accounted for the results obtained in the second half of February where spore counts declined in field 20 but increased in field 31. It is realised that, in the field, fungus growth and sporulation vary with time. For example, in field 20 in January the count was 14 on the 16th, 22 on the 22nd, and 14 on the 27th. Spore counts at approximately weekly samplings may give an inaccurate picture by possibly missing highest or lowest counts that could have occurred in the interval. It is further realised that under favourable conditions at any one time a proportion of spores may have germinated and made sufficient growth anchoring them to leaves and stems and preventing their being washed off and recorded. A decline in spore count caused in this manner may not necessarily reflect a decline in the growth of the fungus.

Facial eczema is a seasonal stock disease which in the past has been associated with the onset of warm autumn rains following summers of above-average temperatures, and the Department of Agriculture has issued precautionary warnings when these conditions occurred. Mitchell, Walshe, and Robertson (1959) have also proposed a scheme of warning for facial eczema based on weather data. An examination was made of weather recordings taken at Manutuke Research Station both of microclimatic data, supplied by Dr K. J. Mitchell, and of the standard data taken for the New Zealand Meteorological Service. No association appeared to occur between spore counts and air temperatures 1 in. above ground, soil temperatures at $\frac{1}{4}$ in. and 1 in. beneath the surface, and soil moisture 1 in. below ground level recorded by gypsum blocks. The response of the latter to moderate rainfall was highly variable in time and only major changes of soil moisture were revealed. Periods of increasing counts indicating active sporulation, in individual fields, could be associated with periods of warm moist conditions in which grass minimum temperatures reached or exceeded 56°F and were accompanied or preceded by light rain totalling 0.50 in. or more (see Fig. 3). Spore counts increased to a maximum after

several successive periods of these warm, moist conditions. However, high spore counts did not occur in every field following the onset of these conditions since nutritional and microclimatic conditions would have influenced fungal growth. Over the period 2-20 February when there was little rain, air temperatures were high and conditions generally were dry, spore counts in fields 20 and 31 remained at a relatively high level and lambs grazing these fields showed clinical symptoms of facial eczema. Later in the autumn growth of the fungus was apparently depressed by unfavourable climatic conditions.

Counts of *S. bakeri* spores in fields 19, 35, and 25 treated with lime and superphosphate and 'dichlone' fungicide were very similar to counts in the control field 10 during the periods under observation (see Table 1). On 24 February, five weeks after treatment, counts from both fields 19 and 35 had increased to a somewhat higher level than those from field 10. This was considered to indicate that high levels of calcium hydroxide or superphosphate did not have any effect upon *S. bakeri* and no further counts were made. Beaker tests, carried out twice weekly until 15 April, did not reveal a significant effect by these treatments. Spore counts from field 25 sprayed with 'dichlone' fungicide increased steadily to 24 March, eight days after the final application of 4 lb per acre. This latter level was thought likely to be well in excess of a level which would poison grazing sheep as Mr I. G. McIntosh (pers. comm.) had shown that one dose of 2.5 g 'dichlone' was fatal to a 60 lb lamb. In this instance no deaths through poisoning occurred.

TABLE 1. INFLUENCE OF LIME, SUPERPHOSPHATE, AND 'DICHLONE' ON COUNTS OF *S. bakeri* IN PASTURES. (FIGURES MULTIPLIED BY 10^6 GIVE SPORE COUNTS PER GRAMME DRY HERBAGE)

Date	Treatment			
	Lime 10 cwt/acre Field 19	Superphosphate 6 cwt/acre Field 35	Dichlone up to 4 lb/acre Field 25	Control Field 10
18 February, 1959	0.75	1.0	0.77	0.69
24 February, 1959	2.4	3.6	2.3	1.5
2 March, 1959	Not determined		1.9	1.2
9 March, 1959	„	„	4.1	3.5
16 March, 1959	„	„	5.7	7.2
24 March, 1959	„	„	4.5	4.5

In making these spore counts on various fields at Manutuke Research Station it was interesting to find that spores of *S. bakeri* were predominant in the washings from pasture samples. Apart from spores of *Fusarium* type, other fungal spores were of infrequent occurrence. This may indicate that *S. bakeri* is an important fungus in the decomposition of pasture litter.

A sample of turnip leaf litter gathered from a 2 acre field adjacent to field 10 on 16 March, and which had been grazed by lambs, did not

contain spores of *S. bakeri*. Eight lambs which had grazed on the turnips for the season did not show liver damage when slaughtered. Face wool on lambs grazing on field 37 was observed to become more grey in colour. A sample of this wool was clipped from several lambs on 25 March and gave a count of about 10×10^5 spores/g dry weight wool. This may provide a quick qualitative field method for assessing the presence of spores of *S. bakeri* in particular areas.

Occurrence of S. bakeri *in Other Locations*

Herbage samples from a pure ryegrass pasture at Palmerston North, maintained by the Plant Chemistry Division, DSIR (Butler 1958, plot 8, paddock 16), were examined on six occasions in March. Counts of between 1.2 and 3.2 were recorded. Sheep grazing on this area, when slaughtered, showed severe liver damage (Dr G. W. Butler, pers. comm.). An area in the same plot which had been closely mown and the clippings left on the pasture for 7 days, gave a spore count of 16 which was a five fold increase. This confirmed the findings at Manutuke that additions of freshly killed or damaged pasture plant material induced a high spore count.

Spores of *S. bakeri* were recorded from pasture samples from the following districts in late February-early March: Waiouru, Hastings, Wairarapa, Hutt Valley, Lincoln, Gore. The incidence was much lower than in samples from Gisborne and Palmerston North. The spore content of samples from South Island was lower than those from North Island, in fact too low for counts to be made. Counts on litter samples from Lincoln, from areas which had been mown on several occasions and the clippings left on the ground, were about 1.2, which were lower than the count obtained on litter from a field at Manutuke. This lower incidence of *S. bakeri* spores in a field with an abundant supply of suitable food material is thought to be due to the lack of favourable climatic conditions.

Facial Eczema and S. bakeri

Fields 20 and 31

Three consecutive grazing trials with lambs were carried out in fields 20 and 31 between 9 January and 28 March 1959, using 5 lambs for each field for each grazing period. The results of these trials are summarised in Table 2.

Lambs were slaughtered at the end of each grazing period. Liver damage was assessed by one of us (D.P.S.) on macroscopic features from experience gained in examining diseased animals over past outbreaks.

Results showed that all lambs in both fields for the first two grazing periods ending 25 February, developed clinical symptoms of facial eczema and when slaughtered exhibited extensive liver damage characteristic of facial eczema disease. Spore counts in these toxic fields were above a mean of 4.

During the third grazing period, 26 February-28 March, there were no clinical cases in either field, and when the lambs were

TABLE 2. RELATIONSHIP OF COUNTS OF SPORES OF *Sporidesmium bakeri* ON PASTURE TO FACIAL ECZEMA DISEASE

Field No.	Grazing Period	Nos. of Lambs Grazed	Spore Count*	Percentage of Lambs Photophobic	Liver Assessment**					
					Mean†	N	1	2	3	4
20 Mow and return	9–27 Jan.	5	16.5 (14–22)	100	36				2	3
	28 Jan.–25 Feb.	5	9.4 (4–14)	100	40					5
	26 Feb.–28 Mar.	5	3.2 (1–5)	0	6	4			1	
31 Mow and remove	9–27 Jan.	5	4.0 (1–6)	100	34				3	2
	28 Jan.–25 Feb.	5	7.3 (5–11)	100	36			1		4
	26 Feb.–28 Mar.	5	6.3 (3–9)	0	20	2		1		2
10 Control set stocked	*14 Jan.–29 Apr.*									
	9–27 Jan.		Present							
	28 Jan.–25 Feb.		0.6 (0.1–1.1)							
	26 Feb.–28 Mar.	10	4.4 (1–7)							
	28 Mar.–29 Apr.		3.3 (0.9–5)	10	30	1	1		3	5
1–5 Rotationally mown and rotationally grazed	14 Jan.– 4 Feb.	10	0.6 (0.3–1.1)	0	18	5	2	2	1	
	28 Jan.–18 Feb.	10	0.6 (0.1–1.1)	0	15	5	1		2	2
	18 Feb.–11 Mar.	10	2.1 (1.7–2.6)	0	28	1	1	1	3	4
	11 Mar.– 1 Apr.	10	2.0 (1.2–3.5)	20	35			1	3	6

* Mean of four determinations made in each field during each grazing period. Counts multiplied by 10^5 give numbers of spores per gramme dry herbage. Figures in brackets show range of counts.

** Liver damage assessment values: N = normal, 1 = very slight damage, 2 = slight damage, 3 = moderate damage, 4 = severe damage. Figures refer to numbers of lambs showing particular degrees of liver damage at slaughter.

† A sort of "weighted" mean for each grazing period was calculated by adding the values obtained by multiplying liver assessment values by the numbers of lambs showing particular degrees of liver damage, where N has value 0, 1 value 1, 2 value 2, etc. For convenience these means were expressed on the basis of 10 grazed lambs.

slaughtered the incidence and degree of liver damage was found to be less than in the previous grazing periods. Although spore counts in both fields had early been above the level of 4, these counts had declined over the grazing period.

The higher incidence and degree of liver damage in lambs on these two fields in the first two grazing periods compared with results in the third period cannot be accounted for by the sum of spores ingested. As the weights of lambs increased during these periods, intake of spores on a body weight basis may also be important. Moreover, there appears to be a possible association between the toxicity and trends in spore counts. Fig. 3 shows that rapid growth and sporulation by *S. bakeri* occurred in both fields in the first two grazing periods. This would have produced much growth and many fresh spores in the pastures. In the third grazing period there would have been less growth and most spores would probably have been those retained in the pastures from earlier periods of fungus growth and would, therefore, have been older and more weathered. The results suggest that possibly old spores may be less toxic than fresh spores. These results suggest also that these pastures appeared to be highly toxic only during periods of very active fungal growth and spore production.

Other Fields

In most other fields ten lambs were grazed continuously for the period 14 January-29 April and were slaughtered on 30 April. In fields 1–5, groups of 10 lambs were grazed for overlaping 21 day periods and then slaughtered.

In field 10, spore counts were relatively low, about 0.6, between 9 January and the middle of February, i.e., corresponding to the first two grazing periods in fields 20 and 31. Lambs grazing this field did not show any signs of photophobia. On other less regularly sampled fields, Nos. 1–5, which had similar low spore counts over the same period, lambs when slaughtered showed only a few cases of mild liver damage.

There was rapid sporulation in field 10 in early March and counts reached 7 on the 16th. One lamb was photophobic and when all 10 lambs were slaughtered at the end of April, examination showed 5 cases of severe liver damage, 3 moderate, 1 slight and and 1 normal. In the less regularly sampled fields 1–5, results indicated that severe liver damage ocurred in March, with two lambs photophobic, when there was rapid sporulation, the highest count being 3.5 on the 24th.

In field 37, where active sporulation ocurred from mid February to mid March and counts rose to 14, 3 clinical cases of facial eczema were observed and liver damage was found to be severe in all 10 lambs.

In fields 19, 35, and 25 to which calcium hydroxide, superphosphate, or dichlone had been added, respectively, one or more clinical cases of facial eczema occurred in the latter half of March and all lambs exhibited severe liver damage. Spore counts on these fields could have followed the trend of counts in field 10 (Table 1).

These results indicate a relationship between counts of spores of *S. bakeri* in pasture and facial eczema in lambs and suggest that where active sporulation occurs and counts reach a mean of 4 and above pastures are likely to be highly toxic. This would not be the lowest level at which liver damage would occur but from these investigations it was not possible to determine the lowest level. The time factor would be important in the development of liver damage at low spore levels with possibly a considerable lag in the appearance of clinical symptoms in lambs.

Spore Intake by Lambs

Assuming that a 60 lb lamb ingests 2 lb dry herbage per day then the approximate order of magnitude of the numbers of spores ingested per lamb in fields 20 and 31 over the different grazing periods can be calculated from the mean spore counts given in Table 2. Over an average grazing period which produced clinical symptoms of facial eczema, i.e., fields 20 and 31 for the first two grazing periods, each lamb would have ingested about 19×10^9 spores. From a sample of air-dry spores grown in the laboratory, Mr R. Q. Packard (pers. comm.) found that 1.914×10^7 spores weighed 1.062×10^{-2} g. The approximate weight of 19×10^9 spores would thus be about 10 g.

Spore Counts and Beaker Tests

The "beaker test" of grass samples developed by Perrin (1959) has been shown by Sandos, Clare and White (1959) to be suitable for use as a test for toxic grass, strong positive tests having a good correlation with the toxicity of the sample.

Percival (1959) has reported that the beaker test substance (m.p. 260°C) is contained in the spores of *S. bakeri*. In the present investigation, beaker tests were carried out on dried samples from fields 10, 20 and 31, from 22 January to 15 April approximately twice a week. Field 37 was sampled from 16 February onward together with other fields. Statistical analysis of spore counts and beaker test results from fields 10, 20, 31 and 37 by W. B. Taylor (pers. comm.) showed that the correlation between spore counts and beaker test values had the value 0.6415 which was significant at the 0.01% level of probability. Mean spore counts were determined for each beaker test value and the correlation between these figures is shown in Fig. 4. Spore counts increased by approximately 1.5 for each beaker test value except for the highest value 7. Here the beaker test apparently reached saturation and was unable to distinguish higher spore counts.

From these investigations it would appear that good growth and sporulation of *Sporidesmium bakeri* in the field depended upon a supply of dead or damaged grass, and warm, moist conditions. Outbreaks of the facial eczema disease will depend upon the above conditions producing a high spore count in the pasture. The part of a field grazed by sheep may also be important, because 10-fold variations were found over the samples taken from a field at any one time. Although both high- and low-sporing strains of the fungus have been isolated from samples collected in the field (Thornton and Ross 1959) it is

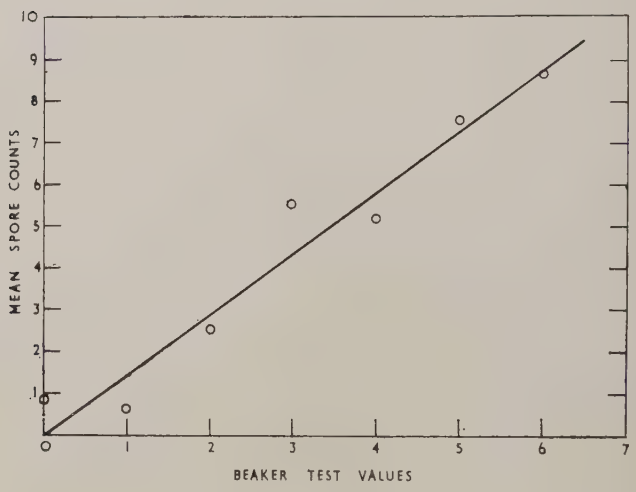

Fig. 4.—Relationship between mean spore counts and beaker-test values.

not known to what extent the prevalence of high- or low-sporing strains might influence the spore content of pastures.

The provision of large quantities of dead and damaged plant material was accomplished in this investigation by mowing and harrowing, but under normal farming conditions suitable dead or damaged plant material may be added under the influence of some other factor. Periods of summer drought could result in the rapid death of tillers while lenient stocking could result in a greater quantity of dead seed-head stalks. The rapidity with which some soils dry out would also cause death of grass tillers. Rapid colonisation of this dead material by *S. bakeri* would occur with the onset of favourable microclimatic conditions.

While none of the treatments described herein appeared to decrease the counts of *S. bakeri* spores, it may be possible by pasture management to reduce the accumulation of litter to an extent sufficient to affect control on the growth of the fungus and reduce the incidence of the disease. Further investigations on these lines appear warranted.

The prevalence of facial eczema disease of sheep in New Zealand, where outbreaks are confined almost entirely to North Island, suggests that its incidence may be related to the type of grassland farming practised and the climatic conditions.

ACKNOWLEDGMENTS

Thanks are due to Mrs E. M. Guy and Miss M. K. Bennett for assistance with spore counts, Messrs B. R. Watkin and G. S. Harris for sending herbage samples, and Mr W. E. Hepburn for carrying out the field treatments at Manutuke Research Station.

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MEASUREMENT OF FEED INTAKE BY GRAZING CATTLE AND SHEEP

VII. MODIFIED WET DIGESTION METHOD FOR DETERMINATION OF CHROMIC OXIDE IN FAECES

By Audrey E. Stevenson and H. de Langen, Ruakura Animal Research Station, Department of Agriculture, Hamilton

(*Received for publication, 17 December 1959*)

Summary

A wet digestion colorimetric method for the determination of chromic oxide marker in faeces is described.

In comparison with other methods examined it has the following advantages:

1. Less critical conditions.
2. Higher reproducibility.
3. Higher recovery.
4. Higher daily output.

Introduction

Increasing use of chromic oxide as a faeces reference substance in herbage intake measurements has made it necessary to investigate a more rapid and reliable routine procedure for estimating chromic oxide than the wet digestion method of Christian and Coup (1954), reported in Part VI of this series.

Methods of several different authors have been examined and assessed as a routine analysis on accuracy, speed, and lack of critical conditions.

A rapid and accurate wet digestion colorimetric method is presented in this paper.

Discussion of Methods

Fusion Methods

The alkaline-oxidation fusion methods of Barnicoat (1945), Kane *et al.* (1950), Schurch *et al.* (1950), Raymond and Minson (1955), and Kameoka *et al.* (1957) were examined.

In fusing with sodium peroxide the loss in weight of the nickel crucible (25 ml) was found to be 2–5 mg per assay. The crucible became pitted and eventually holed in the base and the retention of chromate in the nickel oxide precipitate was appreciable. Both these disadvantages increased with rise in temperature. Temperature deviations of $\pm$ 10°C in the muffle furnace produced differences in results of 5% and more. Fusing over gas burners at low temperature (Kameoka *et al.* 1957) resulted in loss of chromate up to 20% after two minutes melting. At no time was it found that the loss of chromate in the nickel oxide precipitate was a standard one of 2% as described by Barnicoat (1945).

The methods of Kane *et al.* (1950), and Raymond and Minson (1955) developed from that of Edin *et al.* (1945), showed less loss in weight of the nickel crucible. In fusing with a mixture of sodium and potassium hydroxides, together with their carbonates and nitrates in 1 : 2 molecular ratio (Kane *et al.* 1950) the mixture tended to foam and creep over the edge of the crucible and the blank readings were high and variable, giving low results and poor reproducibility. Changing the carbonate : nitrate ratio to 10 : 1 (Sandell 1950) lowered the blank reading, but foaming over still occurred and was found to be due to the nitrates.

Maunsell (1944) stated that fusion with sodium carbonate alone does not bring all the chromic oxide into solution and that a small amount of an oxidising substance must be added to the fusion mixture to convert all the chromium to chromate. It has been found, however, that fusion with sodium carbonate with air oxidation converts all the Cr_2O_3 to the soluble chromite, and partially further to chromate. Hydrogen peroxide readily completes the oxidation. Thus the large expenditure of nickel crucibles and the retention of chromate in the nickel oxide precipitate can be avoided if the oxidising substance is added to the crucible after fusing.

The ashed sample is fused with sodium carbonate over a gas burner, then the crucible is placed in a conical beaker and sufficient hydrogen peroxide solution to cover the melt is added to it and allowed to react. Water is then added to the beaker and the solution boiled to ensure that the chromate is completely dissolved, and to remove excess hydrogen peroxide. After cooling and making up to the mark, the solution is filtered, and read at 400 mμ.

Using 25 ml crucibles of United States origin (weight approx. 20 g, base diameter 2.4 cm) recoveries were high and reproducibility good when care was taken to mix the ash well with the sodium carbonate before fusing, and the method was made routine on a small scale pending a large delivery of crucibles. When the English-manufactured crucibles arrived they were found to be of a thinner mould, weighed approximately 15 g, and had a base diameter of 3 cm. Reproducibility dropped considerably with these and it was thought that the thinner mould was too readily overheated using bunsen burners, with the possibility of chromium silicates being formed. The muffle furnace was then tried, and to overcome the high melting point of sodium carbonate a molecular ratio of 1 : 1 sodium and potassium carbonates was used. Reproducibility seemed to depend essentially on sufficient mixing of the ash containing Cr_2O_3 with the carbonates. A glass rod was used to grind the lumps of ash against the side of the crucible. This time-consuming mixing and the critical heating conditions dependent on the type of nickel crucible available, made it necessary to discard this method for large-scale routine work.

Wet Digestion Methods

The wet digestion method of Christian and Coup (1954), has been in use at this Station since 1954. Reproducibility is high when time and temperature of the digestion are kept uniform, but with excess

heating the oxidising power of the solution is lost and reversion of the reaction takes place. Any effort to increase the daily output of determinations lowered reproducibility and increased between-operator variability, again mainly because of the critical heating conditions (see Table 3).

The wet-digestion method of Coup and Lancaster (1952), described in Part II of this series, was modified by Christian and Coup (1954), mainly because of its low daily output. This was due to the long boiling period necessary because the Cr_2O_3 was not readily attacked by oxidising agents at the temperature of the diluted acid mixture, and also to ensure at a later stage that all the excess bromate was removed for the back-titration. Coup (1952) and Maunsell (1944) stated that it was necessary to keep the volume of the boiling solution constant. It has been found, however, that by digesting with half quantity of the acid mixture of Coup (1952) and one-fifth the amount of bromate solution, and by boiling until the water has evaporated and the sulphuric acid started to fume, the Cr_2O_3 is brought into solution, but not all as dichromate. A further boiling with a small amount of $KBrO_3$ in a diluted solution is sufficient to oxidise all the chromic compounds to dichromate. The presence of sulphuric acid in the acid mixture keeps the temperature below the critical point where dichromate is reduced to insoluble chromic compounds. Thus the critical heating conditions of Christian's modification can be avoided and the long boiling time of Coup's method is reduced to 10–15 minutes. Excess bromate does not interfere when the solution is made alkaline and the chromate estimated colorimetrically.

Chromate solutions obey Beer's law at low concentrations with maximum absorption at wavelength 370 mμ. To cover a wider range of concentrations without dilution, solutions can be measured at wavelength 400 mμ with sufficient accuracy (see Fig. 1).

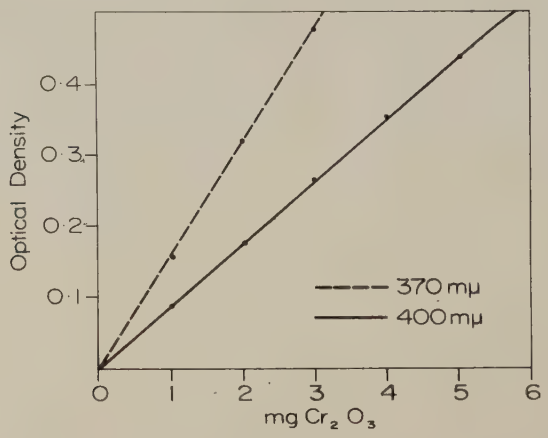

Fig. 1.—Optical density of chromate solutions expressed as Cr_2O_3.

DETERMINATION OF CHROMIUM SESQUIOXIDE IN FAECES

Procedure

The ashed sample containing 1 to 5 mg Cr_2O_3 is quantitatively brushed into a dry 100 ml wide-necked Erlenmeyer flask which is graduated at 100 ml, and 6 ml of acid mixture is added. After bringing to the boil, 3 ml of 4.5% $KBrO_3$ solution is added and boiling continued until ½–1 minute after fumes of SO_3 appear. The flask is removed from the hotplate, allowed to cool, then 20 ml of diluted $KBrO_3$ solution added and the mixture boiled for 3–4 minutes, cooled, made up to the 100 ml mark and mixed. With a pipette, 10 ml is transferred into a 25 ml graduated boiling tube, 5 ml 10% NaOH solution added and heated in a boiling water bath for a few minutes to allow the MnO_2 precipitate to settle. The solution is cooled, brought to volume, and inverted gently once (see Note 4). Sufficient is filtered through a No. 40 Whatman filter paper and the absorption is read at wavelength 400 mμ. A blank is run on Cr_2O_3 free faeces and reagents. For the standard curve a solution of 2.555 g K_2CrO_4/litre is used. (1 ml = 1 mg Cr_2O_3).

Reagents

Acid Mixture:	250 ml conc. H_2SO_4
	250 ml H_3PO_4 (sp. gr. 1.75)
	500 ml distilled water
	50 ml 10% solution $MnSO_4$, $4H_2O$
Dilute Bromate Solution:	50 ml 4.5% $KBrO_3$ solution
	450 ml distilled water

Notes on Procedure

1. Sufficient quantity of $MnSO_4$ must be present, but excess does not interfere. It would appear that the manganous sulphate is oxidised by the bromate to permanganate which in turn oxidises the Cr_2O_3. In Coup's long-boiling method with the acid solution diluted and kept constant at 40–50 ml the $MnSO_4$ acts essentially as a catalyst, but in this method the $MnSO_4$ is more expendable with the formation of MnO_2 precipitate.
2. After the initial digestion by heating until the sulphuric acid fumes, then addition of dilute bromate solution, the volume must not be allowed to drop or reduction occurs. With only 3–4 minutes boiling at this stage there is no need to replenish the water. Boiling for longer periods gives lower results probably owing to reduction of the dichromate as the acid becomes more concentrated. This can be noted by a change in colour from deep brown to milky light brown.
3. Iron and other metals present in the ash help, with heating, to change the MnO_2 precipitate in the alkaline solution from the colloidal form, which passes through the filter paper, to a heavier flocculent precipitate. It is found that the addition of faeces ash to the standard potassium chromate solutions gives a better reproducibility and recovery (see Table 2). The amount of chromate lost in the MnO_2 and other precipitates is negligible.

4. It is important not to *shake* the final solution before reading, as the MnO_2 precipitate is readily broken down again into the colloidal form.

Recovery Tests

Commercial chromic oxide, used in the preparation of capsules, was assayed for purity by this wet digestion colorimetric method (W.D.C.) and that of Christian and Coup (1954). A comparison of the mean results and standard deviations is shown in Table 1 (0.3 g faeces ash was added to each sample).

TABLE 1. PURITY OF Cr_2O_3

Method	Sample	Range %	Mean	No. of Assays	Standard Deviation
Christian and Coup	No. 1–59	96.8–98.2	97.7	8	0.55
W.D.C.	No. 1–59	97.6–99.0	98.2	8	0.55
Christian and Coup	No. 30063	97.1–98.6	98.1	8	0.47
W.D.C.	No. 30063	98.3–99.2	98.7	8	0.38

Table 2 shows results as percentage recovery from samples which were made in the laboratory as follows:

(a) Known amounts of K_2CrO_4 added to dried ground faeces.

(b) Known amounts of K_2CrO_4 solution (2.555 g/litre, 1 ml = 1 mg Cr_2O_3) taken through the digestion procedure.

(c) As (b), but with approximately 0.3 g faeces ash added.

TABLE 2. Cr_2O_3 RECOVERY %

Sample	Mg Cr_2O_3 Assayed				Mean
	2	3	4	5	
a	99.7	102.0	100.4	98.9	100.3
b	99.8	104.0	103.8	98.8	101.6
c	100.7	99.9	99.7	99.8	100.0

Faeces samples from four grazing cows dosed with Cr_2O_3 were assayed in duplicate with the normal daily batch by two different technicians (T1 and T2) to compare between-operator variability on a routine basis. The results are shown in Table 3.

Thus the wet digestion colorimetric method shows greater reproducibility and higher recovery. The daily output can be increased from 36 to 48 estimations without lowering reproducibility which is high to within 2%. The method is recommended as a rapid and reliable procedure for large-scale routine work.

TABLE 3. BETWEEN-OPERATOR VARIABILITY

Sample No.	Christian & Coup		W.D.C.	
	T1	T2	T1	T2
48/300	4.88	4.53	4.80	4.83
50/275	10.11	10.34	10.30	10.42
39/300	5.59	5.31	5.69	5.81
26/318	3.67	3.93	4.13	4.16

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FIELD TRIAL OF EIGHT VARIETIES OF BRASSICA FIELD CROPS IN THE AUCKLAND DISTRICT

I. SUSCEPTIBILITY TO APHIDS AND VIRUS DISEASES

By K. P. Lamb, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(*Received for publication, 4 December 1959*)

Summary

Under field conditions Broad-leaf Essex rape and Superlative swede were highly susceptible to cabbage aphid (*Brevicoryne brassicae*), Wye swede and chou moellier (marrow-stem kale) were moderately susceptible, Doon Spartan and Calder swedes were resistant and York Globe and Green Globe turnips were highly resistant. Varieties did not show the same order of resistance towards *Myzus persicae* as towards *B. brassicae*.

Calder and Doon Spartan swedes and chou moellier were highly resistant to turnip mosaic, while these varieties together with Wye swede were moderately resistant to cauliflower mosaic. The turnip varieties had a high incidence of both virus diseases. Virus disease was the primary cause of plant mortality.

The time of virus infection was correlated with aphid flights. It is suggested that part of the difference in virus incidence was due to vector behaviour.

Chou moellier and Doon Spartan swede were outstanding for winter survival and yield. Calder and Wye swedes were next best, but none of the other varieties was satisfactory under the conditions of this trial.

Introduction

Apart from pasture plants, brassicas are the most extensively grown field crops in this country but the fullest use cannot be made of these crops because of insect pests and diseases which, in some seasons and localities, virtually wipe out susceptible varieties.

One of the most serious insect pests of brassicas is the cabbage aphid (*Brevicoryne brassicae* (L.)) which is responsible for direct feeding damage and also is the main vector of two virus diseases, turnip mosaic and cauliflower mosaic, which are responsible for large annual losses. Some swede varieties (Lamb 1953) are resistant to aphid attack or virus disease in the field and the use of resistant host varieties is potentially of considerable economic importance as a practical method of reducing losses.

Plant breeders and agronomists are constantly introducing new varieties with potential resistance towards other diseases, for example, clubroot and dry rot. It is desirable that the aphid and virus resistance of these varieties should be assessed and taken into account before they are released commercially or used as sources of breeding material.

This field trial compared some of these newer varieties with some known varieties of high and low susceptibility to aphid and virus. Two

varieties of turnips, four varieties of swedes, and one variety each of rape and chou moellier (marrow-stem kale) were tested.

Part 1 of this paper deals particularly with aphid and virus incidence and host mortality. In part II, yield and chemical composition of the varieties will be discussed.

MATERIALS AND METHODS

The following plant varieties were used:

1. Calder swede (aphid and turnip-mosaic resistant).
2. Superlative swede (aphid and turnip-mosaic susceptible).
3. Wye swede (clubroot resistant, newly introduced).
4. Doon Spartan swede (newly introduced variety).
5. Broad-leaf Essex rape (aphid susceptible).
6. Green Globe turnip (standard variety).
7. York Globe turnip (early maturing).
8. Chou moellier.

The plants were sown at the Plant Diseases Division experimental area, Auckland, in six randomised blocks, with three 30–ft rows per plot, on 30 October 1957. All plots were examined and sampled at intervals until October 1958 when remaining plants were flowering and the trial was concluded.

Four Moericke type aphid traps painted with zinc chromate (Lamb 1958) were set up within the trial in November 1957, and weekly aphid catches were counted and identified throughout the trial period.

Unless otherwise specified, aphid counts were transformed to log $(n + 1)$ and subjected to analysis of variance. The equivalent-angle transformation was used for per cent virus-infected plants. Duncan's multiple range test (Le Clerg 1957) was used to compare variety means.

RESULTS

Aphid Trapping

The results of weekly collections are summarised in Figs. 1 and 2.

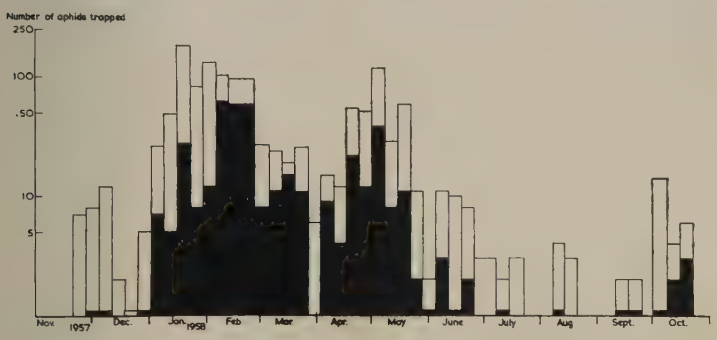

Fig. 1.—Total aphids trapped and *Brevicoryne brassicae* trapped (solid histograms), 1957-58.

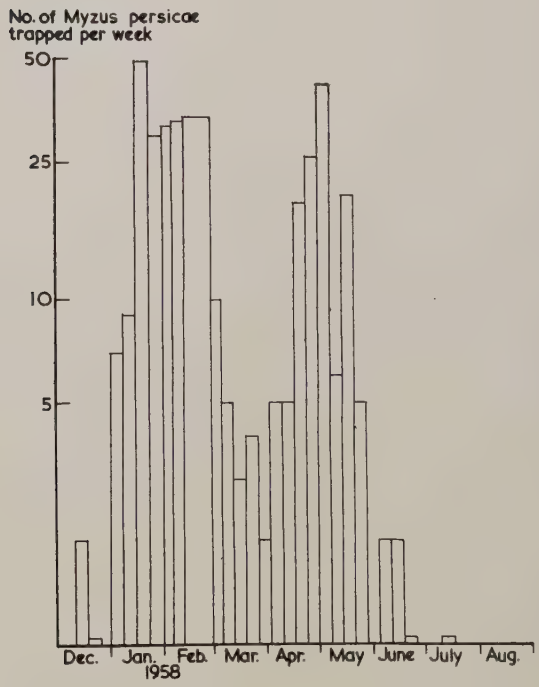

Fig. 2.—Number of *Myzus persicae* trapped, 1957-58.

Brevicoryne brassicae (L.) and *Myzus persicae* (Sulz.) occurred in equal numbers and together comprised 70% of the total aphids trapped. Peak numbers of both species were trapped in January-February. Numbers declined during March, then rose to another peak in late April and early May.

Aphids on Host Plants

Seed had germinated by early November, but few aphids were observed on the plants during November, December, or early January.

On 16 January, aphids were counted on four random plants from each plot and recorded as *B. brassicae* or "other species" (mainly *M. persicae*). The differences between numbers of aphids on some host varieties were highly significant with both sets of counts. The means of these counts are given in Table 1.

By 11 February, aphids were observed in moderate to large numbers on all varieties and rape was showing signs of severe aphid damage. Several days of heavy rain then reduced aphid numbers considerably and on 20 February only small numbers of aphids were found throughout the trial. In the next fortnight, aphid colonies rapidly became re-established and on 5 March numerous aphid parasites and predators were observed (syrphid and cecidomyid larvae, coccinellid adults). At

TABLE 1. MEAN NUMBER OF APHIDS PER PLANT
(First sampling 16 Jan. 1958)

Mean number of B. brassicae *per plant*

Host Variety	no. aphids/plant (as log (n + 1))	Significance*
Broad-leaf Essex rape	1.44	a
Superlative swede	0.93	b
Wye swede	0.76	b
Chou moellier	0.70	b
Doon Spartan swede	0.38	c
Calder swede	0.36	c
York Globe turnip	0.01	d
Green Globe turnip	0.00	d

($F = 20.43$, $P < 0.001$)

Mean number of "other species" of aphids per plant

Host Variety	no. aphids/plant (as log (n + 1))	Significance*
Superlative swede	0.97	a
Calder swede	0.63	b
Wye swede	0.58	b
Broad-leaf Essex rape	0.56	b
Doon Spartan swede	0.35	bc
Chou moellier	0.17	c
York Globe turnip	0.04	c
Green Globe turnip	0.00	c

($F = 8.88$, $P < 0.001$)

* Duncan's multiple range test 5% level. Means followed by the same letter are not significantly different.

this time all plots were rated for cabbage aphid and *Myzus persicae* infestation on the following scale:

1. No aphids.
2. Occasional aphid (i.e. one or two per plant).
3. Few aphids (i.e. scattered individuals but not colonies).
4. Some aphids (i.e. with distinct colonies of aphids).
5. Many aphids (i.e. with massed colonies of aphids).

Because the classes in this rating scale are approximately in geometric ratio, ratings were not transformed prior to statistical analysis. Mean ratings are summarised in Table 2. Differences between varieties were highly significant ($P = 0.001$) for both aphid species.

At this stage both rape and Superlative swedes were heavily infested by *B. brassicae* but there was no substantial change in order of varietal susceptibility compared with the first sampling. There was however a change in order of susceptibility towards *M. persicae*. Wye swedes now supported the largest populations of this species. Aphid populations still remained low on both turnip varieties.

TABLE 2. APHID INFESTATION ON 5 MARCH 1958

B. brassicae

Variety	Mean Rating	Significance
Broad-leaf Essex rape	4.83	a
Superlative swede	4.33	a
Chou moellier	2.83	b
Calder swede	2.67	b
Wye swede	2.50	b
Doon Spartan swede	2.17	bc
York Globe turnip	1.83	bc
Green Globe turnip	1.17	c

($F = 13.7$, $P < 0.001$)

M. persicae

Variety	Mean Rating	Significance
Wye swede	4.00	a
Superlative swede	2.33	b
Doon Spartan swede	1.67	bc
Calder swede	1.50	bc
Chou moellier	1.17	c
Green Globe turnip	1.00	c
York Globe turnip	1.00	c
Broad-leaf Essex rape	1.00	c

($F = 11.91$, $P < 0.001$)

In July all plots were sampled again and final counts of *B. brassicae* were made on all varieties other than turnips which by now were long past maturity and mostly dead. Foliage was in a poor condition by this time, partly through disease and partly as the result of normal seasonal deterioration. The means, shown in Table 3, indicate that the greater part of the winter cabbage aphids were to be found

TABLE 3. MEAN NUMBER OF *B. Brassicae* PER PLANT, AND APHID DENSITY, JULY 1958

Variety	No. Aphids/plant (as log $(n + 1)$)	Aphid Density (as log $(n + 1)$)/ unit plant weight	
Chou moellier	1.19***	0.93	a
Broad-leaf Essex rape	0.30	0.70	ab
Doon Spartan swede	0.21	0.34	b
Calder swede	0.14	0.39	b
Wye swede	0.10	0.23	b
Superlative swede	0.07	0.19	b

($F = 20.07$, $P < 0.001$)
($F = 2.80$, $P = 0.05$)

*** H.S. more than any other variety.

on chou moellier. When the aphid density (log (n + 1) aphids/unit leaf weight) was calculated for the July sampling it was found that the highest aphid densities occurred on rape and chou moellier (Table 3).

From the results shown in Table 1 it was apparent that the plant varieties differed in their relative susceptibility to *B. brassicae* and other aphid species. Statistical analysis of the ratio of numberrs of the two groups of aphids per plant, i.e. (log (n_1 + 1) — log (n_2 + 1)) where n_1 = the number of *B. brassicae* per plant and n_2 = the number of "other species" per plant, showed that the difference between varieties was highly significant. The mean ratios are given in Table 4. The two varieties of turnip were not considered in view of the low aphid numbers on them. These results indicate that both rape and chou

TABLE 4. RATIO OF *B. brassicae* TO "OTHER SPECIES": FIRST SAMPLING

(log (n_1 + 1) — log (n_2 + 1))

Variety	Ratio	Significant Differences
Broad-leaf Essex rape	+0.88	a
Chou moellier	+0.53	ab
Wye swede	+0.18	bc
Doon Spartan swede	+0.03	c
Superlative swede	—0.04	c
Calder swede	—0.27	c

Least significant difference 5% = 0.31
from zero 1% = 0.43

($F = 7.46$, $P < 0.001$)

moellier had a higher proportion of *B. brassicae* to "other species" of aphids. Taking the results of Table 1 into account, this suggests that rape favours *B. brassicae* while the high ratio for chou moellier appears to be due to smaller populations of the other aphid species.

When the relative ratings for *B. brassicae* and *M. persicae* infestation on 5 March 1958 (Table 2) were compared (Table 5), similar

TABLE 5. RATIO OF *B. brassicae* TO *M. persicae* INFESTATION ON 5/3/58

Variety	Relative Rating	Significant Differences
Broad-leaf Essex rape	+3.85	a
Superlative swede	+2.00	b
Chou moellier	+1.67	bc
Calder swede	+1.17	bcd
York Globe turnip	+0.83	cd
Doon Spartan swede	+0.50	cd
Green Globe turnip	+0.17	d
Wye swede	—1.50	e

($F = 11.17$, $P < 0.001$)

results were found. Once again rape was highly favoured by *B. brassicae*, but a striking difference was observed with Wye swede which, at this stage, appeared to be preferred by *M. persicae*.

Plant Diseases and Mortality

By mid December a few plants showed characteristic symptoms of black rot (*Xanthomonas campestris* (Pam.) Dowson) infection on the older leaves. This increased steadily throughout the season until by mid April infection was widespread throughout the trial. Dry rot (*Phoma lingam* (Tode ex Fr.) Desm.) was responsible for a significant mortality of swedes during the winter months (June-August).

On 20 February a few plants showed symptoms suggesting early virus infection. By 25 March virus symptoms were distinct and infection was widespread. Counts were then made of infected plants. No distinction was made between turnip mosaic and cauliflower mosaic. Counts of infected plants of chou moellier and rape are less reliable than for other varieties because of the difficulty of discerning early symptoms on chou moellier and because of extensive aphid damage on rape. The percentages of infected plants were transformed to equivalent angles and subjected to analysis of variance. The means are given in Table 6.

TABLE 6. PERCENTAGE OF VIRUS-INFECTED PLANTS AT 25/3/58

Variety	Mean % Virus Infected	Mean Equivalent Angles	Significance
York Globe turnip	68.2	56.5	a
Green Globe turnip	42.3	40.6	b
Superlative swede	29.4	29.7	bc
Broad-leaf Essex rape	18.7	24.9	c
Wye swede	8.8	19.2	cd
Chou moellier	4.2	9.8	d
Calder swede	3.0	5.5	d
Doon Spartan swede	1.8	5.2	d

($F = 22.33$, $P < 0.001$)

The statistical analysis revealed a significant block effect (F test, $P = 0.01$). Infection was most severe in the most southern block and there was a highly significant gradient of infection declining from south-east to north-west (F test, $P = 0.001$).

There was no corresponding gradient of aphid numbers in either the January or March samplings. This suggests that a few alate aphids probably infected a small number of plants in the most southern block (where symptoms were first observed). If subsequent virus spread was mainly within the trial rather than from outside, a gradient of infection similar to that observed would result.

The turnip varieties, especially York Globe, showed most extensive infection while the percentage of infection in Calder and Doon Spartan swedes and chou moellier was very low.

TABLE 7. COUNTS OF VIRUS-INFECTED PLANTS AT 16/4/58 (H. C. Smith)

Variety	Number of plants with symptoms (2 rows of 20 plants)				Percent infection		
	Nil	TM	CM	TM + CM	No Symptoms	CM	TM
Calder	10	0	10	0			
	20	0	0	0	75	25	0
Superlative	2	0	17	1			
	0	0	13	6	2	93	18
Wye	13	4	0	3			
	12	0	6	2	63	27	23
Doon Spartan	18	0	2	0			
	13	0	7	0	78	23	0
BLE rape	0	0	20	0			
	0	0	20	0	0	100	0
Green Globe turnip	1	4	5	10			
	1	3	3	13	2	78	75
York Globe turnip	1	5	1	13			
	0	9	1	10	1	63	93
Chou moellier	15	0	5	0			
	16	0	4	0	78	23	0
Total		25	114	58			

TM = turnip mosaic; CM = cauliflower mosaic;
TM + CM = mixed infection.

Three weeks later Dr H. C. Smith, a plant pathologist of this Division, examined two rows of twenty plants of each variety and on the basis of leaf symptoms classified the plants as uninfected, turnip-mosaic infected, cauliflower-mosaic infected, or infected with both viruses. Results of these counts are summarised in Table 7. These results indicate that both virus diseases were widespread through the trial and that there were considerable differences in varietal susceptibility towards one or the other virus. To check these visual symptoms, representative samples were taken from plants of each variety and inoculated to tobacco and turnip plants. Results confirmed the visual examination except that turnip mosaic was probably more extensive on Wye and Superlative swedes and Broad-leaf Essex rape than was shown by field observations.

Apart from possibly a small percentage of rape plants, aphids alone were not responsible for death of plants in this season of light to moderate aphid abundance. Fungous, bacterial, and virus diseases on the other hand caused substantial losses of most varieties. Total losses are shown in Table 8 where mean plant numbers per row and plant mortality are given at various times. Plant yield will be discussed in detail in part II of this paper, but to demonstrate the effect of plant mortality on yield, the estimated mean weight of edible plant material per row is given in Table 8.

TABLE 8. PLANT SURVIVAL AND MEAN YIELD (FRESH WEIGHT OF EDIBLE MATERIAL PER ROW) AT VARIOUS TIMES

Variety	25/3/58 No. Plants/row	Yield* (kg)	2/7/58 No. Plants/row	Yield (kg)	Percent Plant Mortality	7/10/58 No Plants Row	Percent Plant Mortality
Calder swede	132	85.0	108	84.2	18	6	95
Superlative swede	122	84.7	56	—	54	7	94
Wye swede	128	100.5	106	80.8	17	17	84
Doon Spartan swede	131	106.0	121	114.7	8	33	75
Broad-leaf Essex rape	123	18.0	68	—	45	3	98
Chou moellier	118	86.3	120	117.1	2	112	5
Green Globe turnip	129	93.5	8	—	94	0	100
York Globe turnip	137	107.5	10	—	93	1	100
	($F < 1.0$)		($F = 27.5$, $P < 0.001$)				

* Calculated from four samples per plot.

There was no significant difference between varieties in the number of plants per row in the sampling of 25 March but in the samples of 2 July the varieties fell into three groups (Duncan's test): Doon Spartan, chou moellier, Calder, Wye > Essex rape, Superlative > York Globe, Green Globe. In the latter sampling there was also a significant decline in plant numbers per row from south-east to north-west (as found with the incidence of virus disease in March). This strongly suggests that virus disease was the primary factor in plant mortality.

DISCUSSION AND CONCLUSION

From these results the varieties may be grouped according to their susceptibility towards *B. brassicae* as follows:

Broad-leaf Essex rape Superlative swede	highly susceptible
Wye swede Chou moellier	moderately susceptible
Doon Spartan swede Calder swede	resistant
York Globe turnip Green Globe turnip	highly resistant

"Susceptibiilty" is measured here by the relative number of aphids per plant at any given time, assuming an equal probability of infection of all varieties by flying aphids. The trial design ensured that all varieties were equally accessible to flying aphids. The subsequent build-up of aphid populations then depended upon such factors as preference, antibiosis, or tolerance (Painter 1951), which are considered to be functions of the plant variety concerned. There is no reason to suppose that susceptibility or resistance are static conditions. Indeed, as Beck (1957) has shown for the European corn borer, host resistance may vary with the age and physiological condition of the plant.

From Tables 1, 2, and 3 it is apparent that several varieties have differing degrees of susceptibility to *B. brassicae* and *M. persicae*. The reason for this is not apparent but it could be linked with the different feeding habits of the two species. Day and Irzykiewicz (1953) showed that the polyphagous species, *Myzus persicae*, has approximately twice the feeding rate of *B. brassicae*. Field studies show that *M. persicae* can thrive on senescent brassica leaves which are quite unfavourable for *B. brassicae*. It therefore appears possible that *M. persicae* can obtain adequate food for survival and reproduction in situations where *B. brassicae* cannot. However, further studies are needed to elucidate these points and such studies would need to take into account behavioural aspects of leaf preference.

The timing and distribution of aphid flights in this trial in general paralleled the pattern observed in this area in the 1956-57 season (Lamb 1958). Broadbent (1957) showed that the time from infection to appearance of symptoms of cauliflower mosaic and turnip mosaic depended upon temperature and was shorter for turnip mosaic than cauliflower mosaic. In the field, in England, cauliflower mosaic symp-

toms appeared four to six weeks after infection in summer. Under glass the time was shorter; the average times being 24 days at 17.5°c and 23 days at 20.5°c for cauliflower mosaic, and 17 and 13 days respectively for turnip mosaic.

In our trial area the mean screen temperatures for January February, and March were 18.8°c, 20.3°c, and 19.6°c respectively. Assuming an incubation period of approximately three weeks for cauliflower mosaic under these conditions, the appearance of extensive virus symptoms in mid March is seen to be correlated with peak aphid flights of both vector species in late January and February. Since no crops of virus-infected brassicas were growing on the experimental area, this extensive appearance of symptoms was probably largely due to spread of infection within the crop. This idea is supported by the observation of a few infected plants in one block on 20 February. It appears probable that virus was taken up by flying aphids from some plants (at least) before symptoms of infection were obvious.

The high incidence of virus infection in turnips is of particular interest in view of the low static aphid populations found on these plants. The low aphid susceptibility of these varieties would be expected to result in increased aphid movement between plants since infected winged aphids would tend to land on a plant, feed, and reject it and fly to another plant—thus increasing virus spread. An infected aphid landing on a suitable host would be more likely to remain there and found a colony and would therefore infect fewer plants.

Both *B. brassicae* and *M. persicae* are known to be vectors of both brassica viruses and since alates of both species were equally abundant up to the beginning of March, it is not possible to determine their relative significance in virus transmission in this trial.

The high mortality of some brassica varieties through virus disease in this trial underlines an important reason for failure of these crops in the Auckland area. From Tables 5 and 6 it may be seen that Calder and Doon Spartan swedes and chou moellier had a high resistance to turnip mosaic while these varieties together with Wye swedes had a moderate degree of resistance to cauliflower mosaic. Chamberlain (1948) recorded the resistance of Calder swede (= Dryland) to turnip mosaic. This was later confirmed in the field by Lamb (1953). Using glasshouse inoculation with cauliflower mosaic, Fry (1952) observed no difference in varietal susceptibility between Calder and Superlative Swedes, Green Globe turnips, or Broadleaf Essex rape in comparison with the other varieties he tested by leaf rubbing. This lends support to the above hypothesis of vector activity as a determining factor in virus incidence in certain host varieties.

Chou moellier and Doon Spartan swedes emerge as outstanding varieties for winter survival and yield (Table 7). Calder and Wye swedes were the next best, while none of the other varieties merit consideration under these rigorous disease conditions.

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DEATH AND REPRODUCTIVE RATES OF SHEEP IN RELATION TO FLOCK CULLING AND SELECTION

By F. Hickey, Wonder Distributors Limited, Feilding

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Summary

The records of a number of commercial sheep flocks, kept in such manner as permitted the collection of reliable data relating to the births, deaths, and other causes of decrement and accretion of flock numbers, have been used to compile tables of mortality, ewe and lamb survival, selection and culling factors, and similar information derivable therefrom. The number of breeding ewes "exposed to risk" totalled 83,113 and observations extended over a period of 6 years, during which 77,493 lambs were born and survived to docking, yielding a mean lambing percentage of 93.9, expressed as the number of lambs docked and marked in relation to the number of ewes mated. Ewe deaths numbered 7,042, and lamb deaths prior to 1½ years of age totalled 8,056, or 10.4% of those docked.

Regression analysis indicated that an equation of the form: log. $q_x = 0.156\ x + 0.24$, where "q_x" is the death-rate per cent at age (x), fitted the basic data with a correlation coefficient of 0.937.

A formula, embodying derived factors relating to the number of lambs docked per ewe mated, the probability of hogget survival to breeding age, and the subsequent mortality year by year of ewes in the breeding flock, was used to illustrate the quantitative relationships which are of importance in the annual casting of ewes for age and in determining the degree of selection of young breeding ewes necessary to maintain a proper balance in the breeding flock between the various decremental and accretional effects.

Introduction

Numerous workers in the field of sheep husbandry have repeatedly drawn attention to the lack of vital statistics appertaining to sheep and have commented upon the difficulty experienced in certain phases of farm management as a consequence thereof. Efficient solution of some of the recurring problems encountered in controlling a flock of breeding ewes demands statistics of a type which will enable predictions to be made relating to the probable future composition of the flock as it will be modified by births, deaths, and sales, for it is upon such factors as these that a decision must be based concerning the number of animals that can be culled and the number of young ewes to be selected to replace them, so that flock numbers may be maintained.

The methods employed in managing a sheep flock vary in some degree from farm to farm but, in general, breeding ewes are "cast for age" when they have reached the stage at which their physical and dental condition, and perhaps their fertility, is waning, while yet being capable of yielding a reasonable price as meat. Apart from the adult or aged ewes cast in this manner, flock numbers are also diminished by the death of animals and by the loss of others which may from time to time be "culled" either for infirmity, undesirable features, or temporary excess of numbers. On the other hand, additions to the average flock

are derived mainly from the birth of lambs and since it is seldom practicable or desirable to admit into the flock all the ewe lambs that survive to breeding age, a greater or lesser degree of selection must be exercised. It is here that appropriate statistics can provide a useful guide.

Genetic considerations may play an important part in determining the number and class of young ewes to be entered in the breeding flock but such matters are outside the scope of the present study. Attention will be confined to those problems which depend solely upon vital statistics for their solution.

Having regard to the paucity of information available relating to the inevitable changes which continuously occur in breeding flocks as a result of births, deaths, cullings, and other losses, it was decided some years ago to enlist the co-operation of a number of sheepfarmers who were known to keep the appropriate records, or were prepared thereafter to do so. It was not possible, for reasons of convenience in supervision, to plan the investigation in such a manner as to give as wide a geographical spread as was originally contemplated, but this does not seem to be of serious import, bearing in mind that lambing percentages, mortality, and farm-management practices vary to such an extent throughout New Zealand that it would clearly not be possible to produce any one set of statistics that would be universally applicable. It was considered that some useful purposes would be served by an analysis of the extensive data collected, and that aid might thereby be provided in the solution of certain common problems. This paper, therefore, records some of the principal findings of the investigation and indicates some of the uses for which data of this nature may be employed.

AGE DISTRIBUTION OF EWES

Over a period of six successive seasons, from 1954 to 1959, inclusive, the relevant records were collected from the co-operating farms where all the lambs born on the properties over the requisite period had been age-marked, and where records were kept in a uniform manner and of such a nature that the number and age-classification of deaths could be unequivocally established. Quite exceptional care was exercised by the many people who co-operated in this investigation and the overall supervision uncovered only an insignificant number of cases which required further elucidation. In the main, the animals involved were cross-bred, but differentiation of breeds was not considered essential to this particular project.

A total of 83,113 breeding ewes came under observation, of whom 82,513 were mated on one or more occasions, and produced a total of 77,493 lambs alive at docking and marking. The "lambing percentage" employed throughout is the number of lambs docked in relation to the number of ewes mated. Table 1 shows the age distribution of the breeding ewes and the number of deaths recorded at each age.

It will be noted that an appreciable number of ewes is included in the higher age brackets, this being due to the practice of two properties which had retained aged ewes for breeding purposes in anticipation of

TABLE 1. AGE DISTRIBUTION OF EWES AND DEATHS AT EACH AGE

Age	1½	2½	3½	4½	5½	6½
Number/Ewes	21,571	19,437	13,552	9,621	7,584	6,193
Deaths/Ewes	654	849	806	827	967	1,115

Age	7½	8½	9½	10½	11½	Totals
Number/Ewes	3,034	1,020	784	234	83	83,113
Deaths/Ewes	774	379	412	176	83	7,042

thereby breeding specifically for longevity! Not all of the animals shown as having died between ages 11½ and 12½ did actually succumb, but as a Table of Mortality must be brought to a finite conclusion at a reasonable point they were all deemed to have died in that year of age.

LAMBING PERFORMANCE

The recorded data relating to lambing performances are summarised and tabulated hereunder:

TABLE 2. LAMBS DOCKED AS PERCENTAGE OF EWES MATED
(Based on 6 years' observations)

Age of Ewes at Mating	Number of Ewes Mated	Number of Lambs Docked	Lambing Percentage	Lamb Deaths Between Docking and Age 1½		Percentage of Lambs (M & F) Surviving to Age 1½
				No.	%	
(a)	(b)	(c)	(d)	(e)	(f)	(g)
1½	21,571	16,869	78.2	2,092	12.4	87.6
2½	19,437	18,348	94.4	2,165	11.8	88.2
3½	13,552	13,118	96.8	1,259	9.6	90.4
4½	9,621	10,506	109.2	1,093	10.4	89.6
5½	7,584	8,236	108.6	626	7.6	92.4
6½	6,193	6,218	100.4	510	8.2	91.8
7½	2,983	2,792	93.6	218	7.8	92.2
8½	931	855	91.8	55	6.4	93.6
9½	479	415	86.6	28	6.8	93.2
10½	162	136	84.2	10	7.2	92.8
Totals/ Means	82,513	77,493	93.9	8,056	10.4	89.6

In regard to the lambing percentages elicited in this study, it is apparent that in general they are somewhat lower than those commonly claimed by authors who have contributed to our knowledge of sheep statistics. In the main, however, the latter have been based upon the experience of research stations and agricultural colleges where the shepherding and general management has probably been of an unusually high standard, and where mating has been conducted on a more intensive scale than is normally practicable on a commercial property.

It has been shown by Inkster (1957) at the Ruakura Animal Research Station, that "extensive" mating is apt to result in a lower lambing percentage, especially on the part of two-tooth ewes, and this was probably a factor contributing to the present results, particularly as the incidence of barrenness was obviously high in ewes of this age, diminishing sharply with the age of the ewe. It is apparent that the reproductive efficiency of ewes could be very considerably enhanced by improvement in management methods and better supervision at mating with, perhaps, hormonal treatment to increase the length and intensity of oestral activity.

MORTALITY DATA

Using the data tabulated in Table 1, a Mortality Table was constructed in a manner similar to that customarily employed in compiling a human life-table from population statistics. In the construction of such a table the first step is to calculate the death rate at each age and this is done by dividing the number of deaths which have occurred in each year of age by the number "exposed to risk" at the same age. Thus, in Table 1, which records the original data derived from the mass of returns from all participating farms, the number of ewe deaths occurring at age $3\frac{1}{2}$ (i.e. between $3\frac{1}{2}$ and attainment of age $4\frac{1}{2}$), is 806, while the number living at age $3\frac{1}{2}$ is 13,552. Hence the death rate per cent is:

$$\frac{806 \times 100}{13{,}552} = 5.95 = q_{3\frac{1}{2}}$$

The death rate (q_x) having been similarly computed for each age, the mortality table is commenced with the arbitrary choice of a number, termed the "radix", to represent the "number living" at the lowest age. In this case, the table commences at age $1\frac{1}{2}$, when ewes are normally bred for the first time, and we commence with the number 1000. The computed death rate at age $1\frac{1}{2}$ is then used to calculate the number of ewes out of the original hypothetical number living at age $1\frac{1}{2}$ which will die before age $2\frac{1}{2}$; the process being repeated year by year until all are dead. It will be seen that from the death rate for each age (col. d) are derived cols. b and c, and these three columns are the essence of a table of mortality, from which many other functions relating to probabilities of death and survival may be computed.

A function, which has no practical application in life contingencies but is normally included as a matter of interest and to facilitate comparisons between one mortality table and another, is the "expectation of life", or the average number of future years lived by a subject now aged (x). It is obtained by dividing the sum of the L_x column from the next highest age to the end of the table by the number living at age (x), thus:

$$e_x = \frac{L_{x+1} + L_{x+2} + L_{x+3} + \ldots}{L_x}$$

But since, on the average, an animal will die in the middle of the year in which its death occurs, it is customary to add 0.5 year to the

ascertained value to give the "complete" expectation of life. This is done in the present table.

It will be noted that the age series: $1\frac{1}{2}$, $2\frac{1}{2}$, $3\frac{1}{2}$, etc., has been used in preference to the integral ages 1, 2, 3 for the reason that the former are the approximate ages at which ewes commence each annual breeding cycle and therefore it is more convenient to base death and survival factors thereon.

TABLE 3. MORTALITY TABLE FOR BREEDING EWES

Age	Number Living	No. Dying Between Age (x) and (x + 1)	Death-rate (%)	Expectation of Life (years)
x	L_x	d_x	q_x	e_x
(a)	(b)	(c)	(d)	(e)
$1\frac{1}{2}$	1,000	30	3.03	6.19
$2\frac{1}{2}$	970	42	4.37	5.36
$3\frac{1}{2}$	928	55	5.95	4.58
$4\frac{1}{2}$	873	75	8.60	3.84
$5\frac{1}{2}$	798	102	12.75	3.15
$6\frac{1}{2}$	696	125	18.00	2.54
$7\frac{1}{2}$	571	146	25.50	2.00
$8\frac{1}{2}$	425	158	37.20	1.50
$9\frac{1}{2}$	267	140	52.50	1.09
$10\frac{1}{2}$	127	96	75.30	0.74
$11\frac{1}{2}$	31	31	100.00	0.50

Regression analysis of the mortality data showed that an equation in the form: $\log.q_x = 0.156x + 0.24$ fitted the curve of deaths with a high degree of fidelity, the correlation coefficient being 0.937, indicating high statistical significance.

A mortality table may be employed for a number of useful purposes, but here it will be used chiefly to develop the concepts underlying the rational application of culling and selection procedures. But it is relevant to refer to another function which in certain circumstances may have some usefulness. It may be desired to compute the probability that a group of ewes of a given age will survive to a later age, and this can readily be calculated as under:

$$ {}_nP_x = \frac{L_{x+n}}{L_x} $$

where "${}_nP_x$" is the probability that a ewe now aged (x) will survive n years. For example, on the basis of this present table of mortality, the probability that an animal of age $3\frac{1}{2}$ will survive to age $8\frac{1}{2}$ is, therefore:

$$ {}_5P_{3\frac{1}{2}} = \frac{L_{8\frac{1}{2}}}{L_{3\frac{1}{2}}} = \frac{425}{928} = 0.458 $$

Hence, the probability is that $4\frac{1}{2}$ out of 100 ewes aged $3\frac{1}{2}$ will survive to age $8\frac{1}{2}$.

USE OF VITAL STATISTICS IN SELECTION OF YOUNG EWES

The selection of young breeding ewes for entrance into a flock, and the culling of animals which it is not desired to breed, is a matter of extreme importance to the long-term successful outcome of the enterprise, and is a task which often poses many problems for the flockmaster. Considerations of general policy, of available labour, of carrying capacity, and of several other factors or combination of factors, may determine the number of animals it is wished to maintain in a flock of breeding ewes, and hence will dictate to some extent the degree of selection to be exercised when the ewe hoggets of the previous season are subjected to selection prior to admission to the breeding flock. But whatever the nature of the selection procedure, whether rigorous or lenient, it is obviously desirable that the operator should be able to determine the effect of a given rate of culling upon the future composition and the productivity of his flock. To do so, he requires reasonably accurate data to guide him whether he proposes to increase, decrease, or merely maintain the number of his breeding ewes.

As a general rule he will probably desire at least to maintain his flock numbers, in which case he will require to balance the number of young ewes admitted to the breeding flock against the number of adult ewes which year by year will either die or be cast for age or for infirmity. Clearly, the number of young ewes that can be successfully reared and introduced as replacements in the flock depends not only upon the number of seasons the breeding ewes are mated but also upon their fecundity and the ability of their ewe lambs to withstand the perils that assail them until they in turn attain breeding age.

NUMERICAL BASIS OF SELECTION

It will be evident that the essential factor required in rationalising the selection procedure is a means of ascertaining the probable total number of ewe lambs that will year by year survive to the mating age, and thus influence the feasible intensity of culling. The number of productive matings that are necessary to enable a ewe to replace herself in the flock is relevant in this connection and therefore it may assist to clarify the picture if we follow the fortunes of a two-tooth ewe to see how many times she must be joined in order to reproduce herself.

We note from col. d of Table 2 that 1,000 two-tooth ewes will on the average produce 782 lambs, and since we can confidently assume that one-half of the lambs born will be female, they will produce 391 ewe lambs. But, apart from col. f of the same table we see that 12.4% of the lambs born to a group of two-tooth ewes will die before they attain age $1\frac{1}{2}$ (when they would normally be mated for the first time), so that the survival probability is 0.876 in respect of each lamb. Therefore, although each individual ewe at its first mating would produce 0.39 ewe lambs, only $(0.782 \times 0.5 \times 0.876) = 0.343$ ewe hoggets would actually survive to the breeding age of $1\frac{1}{2}$ years.

Reference to the Mortality Table (Table 3) indicates that before the second mating, 3 out of every 100 two-tooth ewes will have died,

therefore only 970 out of our original 1000 can be bred a second time and, since at this mating they will on the average each produce 0.944 lambs, with a survival probability of 0.882, we have: $(0.944 \times 0.5 \times 0.882) \times 9.70 = 0.404$ surviving ewe hoggets per ewe mated. Similarly, between the second and third mating, a further 42 of the original 1000 ewes will have died, leaving 928 to be bred a third time and they will produce: $(0.968 \times 0.5 \times 0.904) \times 0.928 = 0.406$ ewe lambs, or a total of 1.153 ewe lambs each as a result of three matings.

In general, we have the equation:

$$\text{"Z"} = \sum_{x=1\frac{1}{2}}^{t} (L_x + Sv_x + \tfrac{1}{2}LP_x)$$

where "Z" = the total number of surviving ewe hoggets resulting from the successive annual matings of each individual ewe,

"L_x" = the probability that a ewe will survive to the mating age (x),

"Sv_x" = the probability that a ewe lamb will attain the breeding age of $1\frac{1}{2}$ years, and

"LP_x" = the lambing percentage, male and female, applicable to a ewe aged (x).

Table 4, which is based on the data yielded by this investigation, brings into a convenient form the survival and lamb-docking percentages by means of which the probable future composition of a flock can be estimated. The source of these several factors is explained in the "notes" which follow the table.

TABLE 4. EWE REPRODUCTION AND LAMB SURVIVAL

No. of Mating	Age of Ewe at Mating (x)	Probability that Ewe will Survive for Mating at Age (x)	Probability that Lamb will Survive to Age $1\frac{1}{2}$	No. of Ewe Lambs Docked (per ewe mated)	No. of Surviving Ewe Lambs (per ewe mated)	Total No. of Surviving Ewe Lambs (per ewe mated) "Z"
(a)	(b)	(c)	(d)	(e)	(f)	(g)
1st	$1\frac{1}{2}$	1.000	0.876	0.391	0.343	
2nd	$2\frac{1}{2}$	0.970	0.882	0.472	0.404	0.747
3rd	$3\frac{1}{2}$	0.928	0.904	0.484	0.406	1.153
4th	$4\frac{1}{2}$	0.873	0.896	0.546	0.427	1.580
5th	$5\frac{1}{2}$	0.798	0.924	0.543	0.400	1.980
6th	$6\frac{1}{2}$	0.696	0.918	0.502	0.321	2.301
7th	$7\frac{1}{2}$	0.571	0.922	0.468	0.246	2.547
8th	$8\frac{1}{2}$	0.425	0.936	0.459	0.183	2.730
9th	$9\frac{1}{2}$	0.267	0.932	0.433	0.108	2.838
10th	$10\frac{1}{2}$	0.127	0.928	0.421	0.050	2.888

Notes: The factors tabulated above are derived from the following sources: col. c from col. b of Table 3; col. d from col. f of Table 2; col. e from col. d of Table 2, divided by 2 to give the number of ewe lambs; col f is the product of cols. c, d, and e, and col. g is the serial summation of col. f.

The above table shows that on the basis of the mortality rates and the lambing percentages recorded in this study, a two-tooth ewe entering the breeding flock requires three successful matings before she replaces herself with a ewe lamb capable of survival to breeding age. And it will be seen that when due allowance is made for the mortality encountered by the breeding ewes and their lambs, the successive mating year by year of the gradually diminishing number of survivors of the original 1000 ewes will produce during 10 years a total of 2888 ewe lambs which will survive to first mating.

THE SELECTION FACTOR

We have now to consider the means whereby a good estimate can be made of the percentage of two-tooth ewes that must be selected and drafted into the breeding flock to maintain existing numbers. The actual degree of selection to be exercised is, of course, profoundly influenced by a number of factors, such as variations in the lambing percentages, in the mortality experienced by both ewes and their lambs, by the policy pursued in casting for age, and by the age composition of the flock as a whole. Moreover, as can be seen from Table 2, not only the lambing percentages but also the chances of lamb survival to breeding age vary with the age of the dam, these chances, on the whole, being improved as the age of the dam at mating increases. It follows, therefore, that if the balance between aged and young ewes in the flock is seriously disturbed, the reproductive performances of the flock, and hence the selection requirements and the culling possibilities, will also be disturbed.

These several factors will doubtless vary from farm to farm throughout the country and, although the overall experience of the large number of sheep considered in this investigation may be generally typical of the situation prevailing on similar properties managed in a similar way, it is unlikely that they would represent conditions on sheep farms in other parts of New Zealand, particularly as there is some interim evidence that one important factor, viz, average lambing percentages, is correlated in some measure with the geographical latitude, and hence with sunlight intensity and rate of change in day length (Hickey 1958).

Selection Factor Based on Vital Statistics

Where the necessary statistical data appertaining to a particular flock or flocks are available, the selection factor ("S") can readily be computed by utilising the function "Z", tabulated in col. g of Table 4, thus:

$$\text{"S"} = \frac{100}{Z}$$

This formula is used to compute the selection factor for determination of the number of replacements required to restore flock numbers when ewes are cast at various ages.

TABLE 5. THE SELECTION FACTOR FOR BREEDING EWES
(Based on Vital Statistics)

	Where Ewes are Cast for Age after the following Number of Matings						
	4	5	6	7	8	9	10
No. of ewe lambs surviving, per ewe mated. "Z"	1.58	1.98	2.30	2.55	2.73	2.84	2.89
Selection factor, "S"	63.3	50.5	43.5	39.2	36.6	35.2	34.6

Selection Factor Based on Empiric Assumptions

In the preceding section we investigated the selection requirements where the probabilities of survival year by year of ewe hoggets and breeding ewes were known with some precision, having been deduced from recorded statistics. In practical flock management, however, the records kept are more often than not inadequate in several respects and consequently only rough estimates can be made of the death rates of ewes and hoggets, and frequently average lambing percentages are also rough estimates and do not discriminate between ewes of different ages. One conventional assumption, widely made, is that the mortality rate affecting the breeding ewes in a mixed-age flock is uniform, whereas it is evident that this cannot be so.

Convenient assumptions of this nature do not take cognisance of the fact that the summation of all the forces which militate against survival are not uniform in operation but increase in intensity with advancing age. Hence, the mortality factor in respect of the breeding ewes will vary in accordance with the age-composition of the flock.

Similarly, the use of a uniform mortality factor for hoggets (for example, 5% or 10%) involves the tacit assumption that the lambs born to ewes of differing ages at parturition are all exposed to the same risk of death before they attain breeding age whereas this, also, is by no means true. Table 2 herein indicates that young breeding ewes are much less successful in rearing their lambs than are aged ewes. For instance, 12.4% of the lambs produced by two-tooth ewes died at some point between docking and the attainment of age $1\frac{1}{2}$, compared with 7.6% of those born to ewes mated at the age of $5\frac{1}{2}$. The causative factors in this situation are many, and do not concern us here, but it is apparent that the age composition of a flock of breeding ewes must have an important influence upon the probable neo-natal mortality of lambs and the likelihood of their survival to breeding age and that only by chance would an arbitrary overall estimate of the death-rate be appropriate.

But, if precise statistics are not available, estimates of longevity are warranted, and indeed unavoidable, and the selection factor ("S") can be computed with reasonable accuracy by means of a simple formula which includes among its terms the average number of matings

per ewe up to and including any particular age. This average assumes that the ewes are mated each year and, since the number of matings reflects the diminution in number of breeding ewes due to mortality, this important factor is thus automatically included. It will be of interest to compare selection and culling factors computed by these means with those derived from the detailed data revealed in this investigation.

The following table shows the number of breeding ewes which survive at each age on the basis of conventional assumptions that 5% and 7.5%, respectively, die or are lost from the flock each year following their first mating at the age of $1\frac{1}{2}$ years.

TABLE 6. THE AVERAGE NUMBER OF MATINGS IN RESPECT OF EVERY 1000 EWES FIRST BRED AT THE AGE OF $1\frac{1}{2}$ YEARS

	Assuming Ewe Deaths at 5% p.a.			Assuming Ewe Deaths at $7\frac{1}{2}$% p.a.		
Age of Ewes	No. of Ewes Surviving to be Mated at Age (x)	Total No. of Matings to Age (x)	Average No. of Matings to Age (x) (per ewe)	No. of Ewes Surviving to be Mated at Age (x)	Total No. of Matings to Age (x)	Average No. of Matings to Age (x) (per ewe)
(a)	(b)	(c)	(d)	(e)	(f)	(g)
$1\frac{1}{2}$	1,000	1,000	1.00	1,000	1,000	1.00
$2\frac{1}{2}$	950	1,950	1.95	925	1,925	1.93
$3\frac{1}{2}$	902	2,852	2.85	856	2,781	2.78
$4\frac{1}{2}$	857	3,709	3.71	792	3,573	3.57
$5\frac{1}{2}$	814	4,523	4.52	733	4,306	4.31
$6\frac{1}{2}$	773	5,296	5.30	678	4,984	4.98
$7\frac{1}{2}$	734	6,030	6.03	627	5,611	5.61
$8\frac{1}{2}$	697	6,727	6.73	580	6,191	6.19
$9\frac{1}{2}$	662	7,389	7.39	536	6,727	6.73
$10\frac{1}{2}$	629	8,018	8.02	596	7,223	7.22

Employing the average number of matings, as per the foregoing table, we can apply the following equation to determine the selection factor ("S"):

$$\text{"S"} = \frac{100}{\text{M} \times \frac{1}{2}\text{LP} \times \text{Sv}}$$

where

"S" = the required selection factor,
"M" = the average number of matings per ewe up to the age when ewes are cast for age,
"LP" = the lambing percentage, and
"Sv" = the factor for hogget survival.

As an example, assume that the average lambing percentage is 95, the hogget death-rate is 10%, and all ewes are culled after rearing the lamb resulting from their fifth mating, then:

$$\text{"S"} = \frac{100}{4.52 \times 0.475 \times 0.90} = \frac{100}{1.932} = 51.75\% \text{ selection of 2-tooth ewes}$$

To facilitate comparison, the selection factor ("S") applicable to a hypothetical range of lambing percentages, ewe-hogget death rate, and breeding ewe deaths, is set out in Table 7.

TABLE 7. THE SELECTION FACTOR WHEN LAMBING PERCENTAGES AND DEATH RATES FOR EWES AND HOGGETS ARE AS UNDER

Lambing Percentage	Ewe Hogget Deaths % p.a.	Where Ewes are C.F.A. after 5 Matings — Selection Factor when Ewe Deaths are:—		Where Ewes are C.F.A. after 6 Matings — Selection Factor when Ewe Deaths are:—	
		5% p.a.	7.5% p.a.	5% p.a.	7.5% p.a.
(a)	(b)	(c)	(d)	(e)	(f)
80	5	58.2	61.1	49.6	52.8
85	5	54.8	57.5	46.7	49.7
95	5	49.0	51.4	41.8	44.5
100	5	46.6	48.9	39.7	42.3
120	5	38.8	40.7	33.0	35.2
80	10	61.5	64.5	52.4	55.8
85	10	57.8	60.7	49.3	52.5
95	10	51.8	54.3	44.4	47.0
100	10	49.2	51.6	41.9	44.6
120	10	41.0	43.0	34.9	37.2

Table 7 clearly illustrates the very powerful effect exerted by the lambing percentage in increasing sheep numbers and in widening the scope for the culling of aged ewes and the selection of young replacements. Compared with this factor, the effect of changes in the death rates of both ewes and hoggets is of relatively minor significance. Thus, a 50% increase in the percentage of lambs docked (from 80 to 120) reduces by 33⅓% the number of two-tooth ewes which must be drafted into the breeding flock to maintain the numbers, whereas a 50% improvement in ewe hogget mortality (from 10% to 5%) increases the culling possibilities by only 5–6%.

The age at which breeding ewes are cast from the flock also has an important influence upon the vital statistics. We can deduce from the foregoing table that advancing the casting age one year results in about 15% fewer replacements being required, but with increasing age of the ewes this figure steadily diminishes as it is offset to some extent by the increasing mortality in older animals. Determination of the optimum age for casting is important in flock management and depends upon several variable factors of which, as we have seen, the lambing percentage is the most potent.

In operating the "selection factor", it should be noted that it represents the proportion of the ewe lamb crop which should be selected to maintain flock numbers on the assumption that all the breeding ewes of a chosen age are to be culled after they have reared their lamb for that season and are not to be bred again. It is also assumed that there will be no other cullings apart from these, all reduction in flock numbers being the result of deaths or missing animals.

It is therefore the *mean* selection rate over the period of years appertaining to the breeding activities of the group; that is, if ewes are to be cast when they have been bred five times and have, on the average, produced five lambs, this period is five years. Its principal use is in long-term planning and in providing the flockmaster with a reliable overall picture of the pattern in which flock numbers will develop.

DISCUSSION

In the absence of more precise information, it has been the custom for authors wishing to illustrate a reasonable basis for culling and the selection of young replacements to assume more or less arbitrary percentages in respect to ewe and hogget death-rates. It is quite commonly assumed that a death-rate of 5% for both ewes and hoggets will fairly represent the mortality of the average New Zealand flock, but the soundness of these assumptions is not supported by the vital statistics revealed in this study. Indeed, one of the outstanding impressions derived from the study is that neo-natal mortality, and the probability that a ewe hogget will survive the period from docking to breeding age, must often be estimated with somewhat undue optimism.

In this investigation, hogget mortality was close to 10%, during years in which "ill-thrift", facial eczema, and other ailments that affect young sheep, did not take undue toll. It is evident that the mortality in the flocks embraced by this study may not be representative of the industry as a whole, probably being lighter in some areas and distinctly heavier in others, and no doubt seasonal variations also exert profound effects, but the data suggest that in all circumstances this factor should be estimated with care.

So far as adult ewes are concerned, it was pointed out earlier that the assumption of a uniform death-rate applicable to all ages is inherently unsound as it masks the increasing force of mortality associated with advancing age. The error is considerable in the case of a flock containing a significant proportion of aged ewes. For instance, it can be seen from Table 6 that if the death-rate among the breeding ewes, irrespective of age, be estimated at 5% per annum, no less than 62.9% of the ewes which entered the breeding flock at the age of $1\frac{1}{2}$ would still be alive at $10\frac{1}{2}$, whereas according to the experience of this mortality study only 12.7% would actually have survived to that age.

Nevertheless, no great error is evidently involved in assuming a uniform death-rate of 5% in flocks where ewes are cast-for-age after 5 matings, since the mean mortality rate over this period was found in this study to be 5.5% per annum, but if breeding ewes are retained to higher ages it would be prudent to anticipate substantially greater annual losses.

From the results of this investigation it can be concluded that empiric assumptions as to death-rates and lambing percentages should not, if well chosen, lead to conclusions materially different from those founded upon more exacting analysis. Therefore, such estimates can be employed in computing selection and culling rates with a degree of accuracy which should be sufficient for all practical purposes, but it is

highly desirable that flockmasters should endeavour to maintain more complete records than is generally the case at present. The accumulation of data from a large number of flocks of differing breeds, geographical distribution, and management practices, could conceivably lead to clarification of some important points which affect the control of flocks and the ultimate financial well-being of the sheep industry.

It should be stressed that throughout this investigation the "lambing percentage" has been expressed as the number of lambs docked in relation to the number of ewes mated, which appears to be the most satisfactory measure of the fecundity of the breeding flock, but far too often the lamb crop is expressed as a percentage of the number of parturient ewes, and this of course obscures the important part played by the incidence of barrenness in the ewes and therefore fails to pinpoint this avenue for improving management techniques.

Perhaps of particular importance at the present time is the desirability that the increasing awareness of the advantages accruing from the regular age-marking of sheep should be still further extended within the industry and that its universal adoption should be the goal. The implementation of such a step would greatly facilitate the assemblage of accurate data and their application to the solution of some outstanding problems.

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INVESTIGATIONS INTO SEED SETTING IN COCKSFOOT SEED CROPS IN NEW ZEALAND

By Margot E. H. Johnston, Seed Testing Station, Department of Agriculture, Palmerston North

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Summary

Surveys in cocksfoot seed producing districts of the South Island showed that seed-setting in cocksfoot was very variable.

A wide range of thrips infestation in the cocksfoot florets was found, usually greater in older stands. The thrips found was identified as *Chirothrips pallidicornis* Priesner.

It appeared that the overwintering thrips laid eggs inside the cocksfoot florets before flowering. The first-generation larval thrips destroyed the floral parts before flowering and the first-generation adults laid eggs in florets at or near anthesis, the second-generation larvae destroying the developing seed. The second-generation adults were thought to overwinter inside florets at or near the base of the cocksfoot plant.

In all material examined, a certain proportion of undamaged cocksfoot florets failed to set seed, this failure being a significant factor in reducing seed yield.

Introduction

The low yield of New Zealand-grown cocksfoot seed crops compared with yields obtained in other seed-producing countries, together with the low pure-seed content of many lines of seed, led to investigations into the flowering and seed setting of cocksfoot.

Preliminary Investigations

In 1950-51 summer, the flowering and seed setting in cocksfoot panicles were studied under glasshouse conditions. Results from individual panicles showed that the seed setting ranged from 21.9% to 62.6%, with an average of 52.4%; the "seed setting" being the percentage of the total number of florets in a panicle that contain mature developed caryopses. Field samples were also collected at harvest time in the Southland and Ashburton districts. The seed setting in these samples varied from 2.7% to 29.4%.

In 1951-52, the flowering and seed setting were studied under field conditions on cocksfoot trials being carried out by the Grasslands Division of DSIR. In these trials three New Zealand strains of cocksfoot, Akaroa, C.23, and Grasslands selection, were being compared for seed yield. The trials were in three areas, Palmerston North, Lincoln, and Gore. The seed-setting studies were carried out at Palmerston North and Lincoln, and material was collected from the Gore trial at harvest. Results of the seed setting in these three trials are shown in Table 1.

Further material was also collected at flowering time in the Christchurch and Ashburton districts and later at harvest time from north,

TABLE 1. Percentage Seed Setting in Three Strains of Cocksfoot, 1951-52

Locality	Strain		
	C.23	Akaroa	Grasslands Selection
Palmerston North	45.5	34.7	46.8
Lincoln	53.1	50.8	51.3
Gore	27.4	50.6	45.1

TABLE 2. Percentage Seed Setting in Cocksfoot Crops from Canterbury and Southland, January 1952

Locality	Percentage of Florets Containing Developed Caryopses
Rangiora 1	42.9
Rangiora 2	41.5
Rangiora 3	45.0
Rangiora 4	31.3
Ashburton	32.9
Timaru	6.0
Eastern Southland 1	52.0
Eastern Southland 2	33.9
Eastern Southland 3	25.3
Western Southland	31.7

mid, and south Canterbury and Southland. The results of the seed settings in the mature samples from these areas are shown in Table 2.

The seed setting as revealed in these two tables shows great variation. For all three strains in the Lincoln trial, for one strain in the Gore trial, and in the eastern Southland No. 1 area, it was over 50%, whereas only 6% was found in the sample from the Timaru district. In this sample 27.9% of the florets contained thrips. Thrips were also found in the material collected near Christchurch and Ashburton at flowering time. In these samples the number of thrips found varied greatly from sample to sample, as is shown in Table 3. Variable infestations of thrips were also observed in the mature field samples in both 1950-51 and 1951-52 seasons.

TABLE 3. Thrips Found in Cocksfoot Crops in Canterbury, December 1951

Locality	Percentage of Florets Containing Thrips
Wakanui 1	21.1
Wakanui 2	2.0
Ashburton	2.3
Eiffelton 1	19.0
Eiffelton 2	26.7
Ellesmere 1	3.4
Ellesmere 2	2.7

As these preliminary investigations had shown very variable seed settings and the possibility of widespread thrips infestation, it was decided to carry out a survey of cocksfoot seed crops in the South Island.

MATERIAL SURVEYED

In the 1952-53 season, samples were collected from forty seed stands and from three seed production trials in seed-growing districts in the South Island. The dates of collection, the districts visited and the number of samples collected in each district are shown in Table 4. Just prior to harvest, further samples were collected from some of the same areas in the Rangiora, Christchurch, Ashburton, and Timaru districts.

TABLE 4. CROPS SAMPLED, 1952-53

Date of Collection	District	Number of Crops Sampled
Dec. 1– 4, 1952	Rangiora	7
Dec. 5, 1952	Banks Peninsula	3
Dec. 8, 1952	Christchurch	5
Dec. 10–17, 1952	Ashburton	9
Dec. 19–24, 1952	Timaru	6
Jan. 5– 6, 1953	Eastern Southland	6
Jan. 7– 9, 1953	Western Southland	7

In January 1954, eleven areas in Canterbury and three stands from one farm in western Southland were sampled.

Methods of Sampling and Examination

In each stand, a sample of panicles was collected at random through the stand. In addition, in some crops the density of the stand was estimated by making panicle counts per unit area. As soon as possible after collection the material was examined. Ten panicles (Jan. 1954, 15 panicles) were taken at random from each sample and any one of the five lower stems was removed and examined microscopically (6× of 10× mag.). Each spikelet was dissected, beginning at the base of the stem. The condition of each floret in a spikelet was recorded.

Description of Material Sampled

Of necessity, the samples collected were in varying stages of maturity. The samples from the Rangiora district had not begun to flower; the samples from Banks Peninsula, Christchurch, Ashburton, and Timaru varied from unflowered panicles to panicles in full flower. The Southland samples, collected after an interval of twelve days, were nearly mature. The second and third series, collected in Canterbury in January 1953 and in January 1954, were mature. The varying stages of maturity led to slight differences in the classification of the florets when examining the material.

The Cocksfoot Panicle

The cocksfoot panicle consisted of clusters of spikelets arranged on stems or branches, which became smaller near the tip of the panicle. The spikelet of a normal cocksfoot panicle was found to be made up of usually two to five florets arranged alternately. The innermost floret was variable, sometimes fertile, sometimes consisting of palea and lemma only or of lemma only. All other florets in the spikelet were fertile. The lowest or outermost floret was always furthest ahead in development. A fertile floret before flowering appeared normal, hermaphrodite, with developing ovary and stigma, and three stamens with ripening anthers. In the flowering floret the anthers were exserted, sometimes blown away, the filaments were visible, the ovary and stigma were large. After the floret had flowered, the filaments disappeared and the stigma withered. As the panicle matured, caryopsis development continued; some ovaries though apparently fertilised did not continue development into mature seeds. In mature panicles, these florets contained shrivelled caryopses at various stages of development. In many cases there was no development of the ovary after flowering, probably owing to lack of fertilisation leaving the floret apparently empty. In mature samples a few apparently normal unflowered* florets were occasionally found.

Insects and Abnormalities Observed

Thrips. Thrips were observed first in florets on cocksfoot panicles which had not begun to flower. Florets were observed containing anthers which appeared to be shrivelling and drying up. On closer examination, small shining transparent pale greenish-white larval thrips were seen. These thrips appeared to be feeding on the developing floral parts, causing shrivelling and drying. In more mature material, the thrips larvae were larger and bloated looking. Many florets were also found in which the floral parts were dried up and shrivelled, typical of thrips damage, but in which thrips were not seen. In recording the thrips infestation, these damaged florets not containing thrips were grouped separately. The presence of remnants of shrivelled anthers in florets, with or without thrips, showed that the infestation and damage had taken place before flowering, so that it was possible to distinguish between unflowered and flowered florets containing thrips.

As more mature material was examined, adult thrips were found in unflowered florets. These adult forms were found in four samples in the first series from Canterbury and in all but two samples from Southland. Larval thrips were found also in flowered florets in all samples from Southland. In the fully mature material collected in Canterbury in January 1953 and 1954, larval and adult thrips were observed in all stages of floret development. The adult thrips were black with fawn coloured wings. Black wingless adults were also found.

*This term is used in text and tables for:
A florets examined before flowering;
B florets examined after time of flowering that have failed to flower for no apparent reason.

The first determination of the thrips was made by Mr K. M. Doull, Lincoln College, as *Chirothrips manicatus* Hal. Later material was sent to the Commonwealth Institute of Entomology where the specimens were identified as *Chirothrips pallidicornis* Priesner.

Other thrips were found, including a yellow-coloured, free moving form which was found in florets before and after flowering. It was also observed moving freely over the outside of the panicle and inside the leaf sheath. This form appeared to be wingless at all stages. Colourless and grey coloured adult thrips, thought to be variants of *C. pallidicornis,* were also found.

Other Abnormalities. Nematode galls were found occasionally replacing floral parts in samples from most areas. One sample contained 6.8% of florets with nematode galls. In a few samples traces of cocksfoot midge were found.

Other abnormalities observed were ergot sclerotia, insect eggs (unidentified) found inside florets, traces of other insect damage and a sporadic appearance of brown withered florets, now known to be due to Rathays disease (Johnston 1956).

AN ABNORMAL CONDITION OBSERVED IN SOME UNFLOWERED FLORETS

Some samples, from Banks Peninsula and mid Canterbury, showed an abnormal condition of the anthers in some unflowered florets. In the innermost fertile floret and sometimes the floret next to it, the anthers occasionally appeared to be abnormal, having a yellowish flattened waxy appearance differing from the more greenish plump dry look of normal anthers. The condition appeared to be sporadic. All or almost all of the spikelets in a stem would be affected and the next stem might not show any trace of the condition. The more mature material from Southland and the samples collected in January 1953 showed only traces of the condition, the anthers then appearing to be dried up. Traces only have been observed in the later seasons.

RESULTS OF EXAMINATIONS

The crops sampled fell into several groups according to the stage of development of the cocksfoot panicle. The categories of classification changed from group to group as different features emerged with increased maturity. Details of the samples and groups were:

Group 1. *Sampled before flowering,* from 1 to 4 December 1952, 7 crops all in north Canterbury.

Group 2. *Sampled during flowering,* from 8 to 22 December 1952. There were 23 crops in this group from the Christchurch district, Banks Peninsula, mid and south Canterbury.

Group 3. *Sampled after flowering,* from 5 to 8 January 1953, 13 crops from Southland.

Group 4. *Sampled before harvest,* when mature. All the north Canterbury crops in group 1, and 10 of the crops in group 2 were resampled in January 1953.

Group 5. *Sampled when mature.* A further set of 14 crops were sampled a year later, in January 1954, in mid Canterbury and Southland.

In all groups the two main categories were:

A. Hermaphrodite florets

B. Sterile florets

The hermaphrodite florets were further classified into:

Group 1.	1. Undamaged. 2. Containing thrips. 3. Showing evidence of thrips damage, thrips not found. 4. Containing nematode galls, midges or other abnormalities.
Group 2.	1. Undamaged. 2. Unflowered, containing thrips. 3. Flowered, containing thrips. 4. Unflowered, showing evidence of thrips damage, thrips not found. 5. Unflowered, anthers apparently not normal. 6. Containing nematode galls, midges, etc.
Group 3.	1. Undamaged, containing developed caryopses. 2. Undamaged, containing undeveloped caryopses or empty. 3. Flowered, containing larval thrips. 4. Unflowered, containing larval thrips. 5. Unflowered, containing adult thrips. 6. Unflowered, showing evidence of thrips damage, thrips not found. 7. Containing nematode galls, midges, etc.
Groups 4 and 5.	Same as for Group 3, but a further category "flowered, containing adult thrips" was necessary.

DISCUSSION

As the number of sterile florets in cocksfoot would not be affected by the flowering stage of the panicle, the percentages found in all samples were considered together. In the material examined, the range of sterile florets was as follows:

Samples taken before flowering	6.7%–12.4%
Samples taken during flowering	6.9%–16.0%
Samples taken after flowering	5.4%–18.4%
Samples taken at harvest 1953	5.7%–15.1%
Samples taken at harvest 1954	8.5%–14.2%

The mean percentage of sterile florets for all samples was 10.5%. For all samples, therefore, the mean percentage of fertile florets would be 89.5%. Under theoretically ideal conditions where every fertile floret flowered and was pollinated, and every fertilised ovary continued development into a mature seed, 89.5% of the florets would produce seed. This ideal is never realised. From the results of the observations made in the surveys, failure to reach the theoretical maximum seed setting appeared to be due to the following causes:

1. Damage due to nematodes, midges, other insects, ergot, other diseases, and the occurrence of florets containing abnormal anthers.
2. Infestation by thrips.
3. Some unexplained failure to set seed or for seed to develop.

1. *Damage due to nematodes,* midges, other unspecified insects, ergot, and other diseases and florets containing abnormal anthers.

In the material examined from the surveys, the damage caused by nematodes, etc., was as follows:

Stage at which Samples Taken	Range of Damage Caused
Before flowering	Nil– 6.8%
During flowering	
a. Nematodes, etc.	Nil– 0.9%
b. Florets containing abnormal anthers	Nil–27.3%
After flowering	0.3%– 5.2%
At harvest 1953	0.1%– 7.3%
At harvest 1954	Nil– 2.1%

From these figures, it would appear that the damage due to nematodes, midges, other unspecified insects, ergot and other diseases was of little importance. Florets containing abnormal anthers occurred in larger numbers in some of the crops of group 3 (sampled during flowering). These florets comprised 35%, 27%, 25%, 16%, and 11% of all florets in the 5 worst crops. Other crops in group 3 had less than 8%. Apart from one crop in group 4, which had 5% of such florets, they occurred in only negligible numbers in the crops in groups other than group 3. The reason for this abnormality is not known, or why it should appear only in crops sampled during flowering.

One crop in group 4 had 7% unflowered florets with normal anthers.

2. *Damage Due to Thrips*

The damage in the cocksfoot panicle due to the thrips was divided into two parts, the florets actually containing thrips and unflowered florets which showed evidence of thrips damage but in which thrips were not found. Table 5 shows the range of total thrips damage, within each flowering group.

TABLE 5. RANGE OF THRIPS INFESTATION AND DAMAGE

Stage of Development of the Cocksfoot Panicle	Unflowered Florets		Flowered Florets		Damage Due to Thrips but Thrips not found (%)	Total Damage Due to Thrips (%)
	Larval Stage of Thrips (%)	Adult Stage of Thrips (%)	Larval Stage of Thrips (%)	Adult Stage of Thrips (%)		
Before flowering	Nil– 3.5	Nil– 0.1	—	—	0.1–11.0	0.1–11.4
During flowering	0.4–14.9	Nil– 0.3	Nil– 0.9	—	0.5–27.5	1.0–42.5
After flowering	0.1–19.4	Nil–10.0	0.3–10.0	—	0.3–27.6	1.2–63.3
At harvest, 1953	Nil– 2.1	Nil–12.6	Nil– 8.6	Nil– 7.6	Nil–36.5	Nil–58.2
At harvest, 1954	Nil– 0.9	0.1–28.7	0.4– 3.3	Tr–11.9	0.4–18.8	1.5–53.1

In group 1 (samples taken before flowering), the thrips damage was not great. Undamaged florets comprised more than 76% of all

the hermaphrodite florets in all the samples. One sample had as many as 3.6% of the florets containing thrips, and 5.3% showing evidence of thrips damage. Another sample had 11% of florets showing thrips damage but only 0.4% of florets contained thrips. The former sample was the only one which contained any adult thrips.

In group 2, increasing numbers of thrips were found. The percentage of hermaphrodite florets which were unflowered and contained thrips were:

Below 5% of the florets	14 crops
5–10% of the florets	3 crops
10–15% of the florets	6 crops

Only in 3 of these crops were any adult thrips found and then they were few in number. Thrips were found in flowered florets in 6 crops, but all in amounts of 0.9% or less. These thrips were all the larval stage. It was only the last five crops sampled which showed these thrips in the flowered florets; these crops were probably at a more advanced stage of flowering. Some crops had considerable numbers of unflowered florets showing evidence of thrips damage but in which thrips were not found. These were usually the crops which also had larger amounts of florets in which thrips were found.

The spread in these crops was:

With under 5% of florets showing evidence of thrips damage	11 crops
5–10% „ „ „ „ „ „ „	5 crops
10–20% „ „ „ „ „ „ „	3 crops
20–30% „ „ „ „ „ „ „	4 crops

In group 3, which was sampled after flowering, greater numbers of thrips and more thrips damage were found. There were 2 crops, one at Benmore and one at Greenvale, which showed markedly more thrips than all the others, and the results are summarised in Table 6.

At this stage more larval thrips, but not adults, were found in flowered florets.

In the crops sampled before harvest in both years, larval and adult thrips were found in unflowered and flowered florets, the number of larvae found having decreased. In group 4, five crops showed more evidence of thrips damage than the others, as shown in Table 7.

In group 5, the "flowered florets containing larval thrips" percentages were consistent for all crops, between 0.4% and 3.3%: likewise, the "flowered florets containing adult thrips" percentage, trace -6.6%, except for one crop with 11.9%. For "unflowered florets containing larval thrips", values only ranged up to 0.9%. For "unflowered florets containing adult thrips", there was a wide spread:

Under 4%	5 crops
9–10%	2 crops
15%	1 crop
20–30%	6 crops

The picture was similar for "unflowered florets showing evidence

TABLE 6. DATA RECORDED FOR GROUP 3

Crop Locality	Percentage of Hermaphrodite Florets							Percentage of Sterile Florets
	Undamaged		Flowered	Unflowered				
	Containing Developed Caryopses	Containing Undeveloped Caryopses or Empty	Containing Larval Thrips	Containing Larval Thrips	Containing Adult Thrips	Showing Thrips Damage. Thrips Not Found	Containing Galls, Midges, etc.	
Benmore	7.2	14.0	10.0	15.7	10.0	27.6	2.0	13.5
Greenvale	23.7	21.9	1.9	19.4	5.5	15.5	2.3	9.8
Others	31.4–64.8	21.9–59.5	0.3–4.4	0.1–5.2	Nil–2.6	0.7–7.7	0.3–5.2	5.4–18.4

TABLE 7. DATA RECORDED FOR GROUP 4

Crop Locality	Percentage of Hermaphrodite Florets								Percentage of Sterile Florets
	Undamaged		Flowered		Unflowered				
	Containing Developed Caryopses	Containing Undvlpd Caryopses or Empty	Containing Larval Thrips	Containing Adult Thrips	Containing Larval Thrips	Containing Adult Thrips	Showing Thrips Damage. Thrips Not Found	Containing Galls, Midges, etc.	
Ellesmere	23.4	33.2	6.6	7.6	1.5	8.7	10.9	1.0	7.1
Eiffelton	24.7	29.7	3.6	3.2	1.8	12.6	13.6	0.8	10.0
Claremont	6.3	20.6	8.6	5.7	2.1	8.3	33.5	1.6	13.3
St. Andrews	29.5	33.2	2.8	5.3	0.5	6.1	12.1	0.8	9.7
St. Andrews	1.0	34.2	0.1	0.7	1.1	10.7	36.5	0.6	15.1
Others	26.8–38.5	41.6–59.7	Nil–2.2	Nil–2.8	Nil–0.5	Nil–3.8	Nil–2.7	0.1–7.3	5.7–13.1

of thrips damage, thrips not found" and there was a very close relationship in individual crops between these last two factors.

The maximum total damage in these two "before harvest" sampled groups was 58.2% in the Claremont crop in 1953 and 53.1% in a crop at Benmore in 1954.

The results obtained in these surveys have shown that the damage caused by the thrips was very serious in certain crops. Where the age of the stand was known, the crops have been grouped according to their age as follows:

Age of Stand	1–3 years	4 years or over
Total thrips damage	Nil–5.9%	1.5–58.2%
	(12 crops)	(32 crops)
Mean percentage damage	2.3%	22.0%

It appears from these figures that the degree of thrips infestation is related to the age of the stand. Though in many cases the thrips population was higher in older stands, there were some exceptions of some low infestations in older crops. At the time of sampling, remains of stacks and threshed straw from previous harvests were seen in certain crops and these particular localities were found later to have high thrips populations.

From the results obtained, certain assumptions can be made on the life history of the cocksfoot thrips. It is thought that eggs are laid by overwintering thrips inside the developing florets possibly at the time of panicle emergence. The eggs hatch and minute larvae begin feeding on the developing floral parts, destroying them and preventing the flowering of the floret. Just before flowering, the developing anthers and ovaries are quite large and would provide food material for the growing insect. As the florets approach the flowering stage the larvae become larger and very bloated. It is thought that, during flowering, the adult form appears and emerges from between the pales of the floret in which it has hatched and grown. This adult form probably lays eggs in an open floret at anthesis or shortly after. The egg hatches and the larva feeds on the fertilised ovary, arresting further development of the seed. The larva grows and the second generation adult appears. This stage is probably the next overwintering form which would remain inside the pales of the floret and fall to the ground in or near the base of the plant.

These assumptions are based on the appearance of the small larvae, which were found in unflowered florets in north Canterbury and in the increasing size of the larvae found in the partly flowering samples, with the appearance of the adult stage in the more mature material. This is followed by the finding of the larval stage in flowered florets in the material collected after flowering and finally by the appearance of the adult form in the flowered florets collected at harvest time, together with the decreasing number of larvae, assuming that the larvae have developed into the adult forms.

The observations made in this survey do not agree with those of Doull (1956), who considers that the eggs of the thrips are laid in the

cocksfoot floret at flowering time and not before. However, further observations made in later seasons confirm the examinations made during the surveys and reported above.

Thus the thrips damage occurs in two stages, firstly destruction of the floral parts (preventing the flowering of some florets), and secondly the arresting of seed development after flowering. Although it was always possible to record damage to florets before flowering even without finding the thrips, it was not possible to be certain that damage in flowered florets was due to thrips unless thrips were found. Therefore a certain amount of thrips damage after flowering may not have been recorded.

3. *Failure of Certain Fertile Florets to Set Seed or for Seed to Develop*

The percentage of undamaged hermaphrodite florets that contained developed caryopses and those that were empty or contained undeveloped caryopses were recorded in groups 3, 4, and 5. In crops with less than 10% total thrips damage the ranges of percentages of florets containing developed caryopses were as follows:

Group 3	37.1–64.8%
Group 4	26.8–38.5%
Group 5	28.6–43.9%

In group 5, there were only 5 samples with less than 10% total thrips damage.

It can be seen from these figures, that a large proportion of the hermaphrodite florets fail to set seed. The apparently undamaged hermaphrodite florets without seed, expressed as a percentage of the sum of all the apparently undamaged hermaphrodite florets are shown in Table 8.

The range of the percentages of undamaged hermaphrodite florets without seed within the three groups is as follows:

After Flowering 1953	Before Harvest 1953 (excl. Nos. 27 and 30)	Before Harvest 1954
26.1%–66.0%	51.9%–64.8%	44.0%–65.0%

The range of percentages of apparently undamaged hermaphrodite florets without seed in the samples taken after flowering in January 1953, was 26.1%–66.0%. The figures for samples 31, 32, 33, and 40 were considerably lower than the remaining samples. The percentage seed setting in these four samples was higher than the other samples in the same group. The range of percentages in the samples taken before harvest, 1953, excluding samples 27 and 30, was 51.9%–64.8%. In samples 27 and 30, the seed setting was very low and the amount of thrips damage before flowering was extremely high. In these samples, there was probably considerable thrips damage to flowered florets, which was not recorded, these possibly damaged florets being recorded with apparently undamaged ones, without seed. In the third group of samples taken before harvest 1954, the range was 44.0%–65.0%. For the three groups the upper limits of the ranges were reasonably constant.

TABLE 8. DATA RECORDED FOR GROUPS 3, 4, OR 5

After Flowering 1953			Before Harvest 1953			Before Harvest 1954		
Sample No.	Percentage of Total Undamaged Florets	Undamaged Florets Without Seed Expressed as a Percentage of All Undamaged Florets	Sample No.	Percentage of Total Undamaged Florets	Undamaged Florets Without Seed Expressed as a Percentage of all Undamaged Florets	Sample No.	Percentage of Total Undamaged Florets	Undamaged Florets Without Seed Expressed as a Percentage of all Undamaged Florets
31	87.7	26.1	1	91.6	64.1	1A	56.6	55.3
32	79.4	32.2	2	89.8	66.3	2A	31.3	64.5
33	73.2	29.9	3	83.5	67.9	3A	81.2	61.5
34	45.6	48.0	4	91.5	58.7	4A	42.0	57.1
35	90.9	65.5	5	79.1	58.2	5A	54.7	58.1
36	71.5	47.7	6	84.2	59.7	6A	66.6	52.7
37	86.2	49.4	7	80.3	59.9	7A	85.7	48.8
38	90.1	51.5	13	56.4	58.7	8A	85.2	50.9
39	73.8	51.8	14	84.8	57.1	9A	77.5	44.0
40	89.6	38.3	16	85.2	59.5	10A	45.4	65.0
41	76.3	51.4	18	54.4	54.6	11A	76.5	62.6
42	21.2	66.0	20	80.1	51.9	12A	47.0	51.5
43	84.2	49.6	24	85.4	58.2	13A	45.1	59.0
			25	92.1	64.8	14A	38.2	57.6
			27	26.9	76.6			
			29	62.7	53.0			
			30	35.2	97.2			

Six samples of cocksfoot collected from seed stands in England were obtained and examined similarly to those in the surveys. Expressing the hermaphrodite apparently undamaged florets which failed to set seed as a percentage of the sum of the undamaged hermaphrodite florets, the range is 45.1–65.9%. This is of the same order as the figures obtained for New Zealand-grown cocksfoot.

It would appear that it is the failure of these fertile florets to set seed that is the most significant factor in reducing seed yields.

CONCLUSIONS

Seed setting, and consequently seed yield, in cocksfoot crops is very variable.

One of the causes of low seed setting is shown to be infestation by thrips. The degree of thrips infestation is variable, older stands usually having a higher thrips population.

The investigations disclose that some unknown factor apart from thrips infestation has a marked effect on the percentage of hermaphrodite florets that fail to produce fully developed caryopses.

A percentage of florets is sterile and incapable of caryopsis development under any conditions.

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FRENCH-BEAN RUST (*Uromyces appendiculatus*) STUDIES ON RESISTANCE AND DETERMINATION OF RUST RACES PRESENT IN NEW ZEALAND

By D. E. Yen, Crop Research Division, and R. M. Brien, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

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Summary

The probable races of bean rust present in New Zealand are 10, 17, and 28, as identified in United States of America, with possibly another unidentified race which attacks the differential line "U.S. 780".

In crosses of the resistant variety Westralia with susceptible varieties, heterozygote plants show a delayed, but definite infection with rust in field and glasshouse trials. Homozygous susceptible plants show equal susceptibility with susceptible parents. Susceptibility can thus be termed recessive or dominant according to the time of recording the disease symptoms in segregating populations.

The usefulness of this heterozygote reaction for selection in breeding material is discussed.

Introduction

The pole-bean variety Westralia, has been grown for late-season production in the Auckland area of New Zealand where bean rust, *Uromyces appendiculatus* (Pers.) Unger, is prevalent in late crops (Brien and Jacks 1954). This variety, selected for rust resistance in Western Australia (Cass-Smith *et al.* 1951), has been a successful introduction, and has never shown susceptibility to rust in market crops or in laboratory tests. In other features, however, it does not compare favourably with the local commercial variety Fardenlosa, and thus a breeding project to combine the desirable characters of both in a single variety was commenced in 1953.

Wingard (1933) demonstrated a single-gene difference between resistant and susceptible varieties of pole and dwarf beans, with resistance dominant. Following on their work in the differentiation of twenty physiologic races of *U. appendiculatus,* Zaumeyer and Harter (1941) published the modes of inheritance of resistance to six rust races in four different crosses involving six varieties of beans. While resistances were dominant, several varying explanations of results were offered, depending on the rust races. Single-factor differences for resistance to races 1 and 2 were postulated, while more factors were thought to be involved in resistance to races 6, 11, 12, and 17. Incomplete dominance of resistance to races 11 and 17 was recorded.

The authors pointed out that these results were obtained from observations made on the reaction to rust races at the seedling stage only.

In the material reported on here, the F2 from the cross Westralia × Fardenlosa, inoculated with a mixed sample of New Zealand rust races, gave seedling reactions under conditions similar to those

described by Zaumeyer and Harter, which indicated that resistance was controlled by a dominant gene. Resistant seedlings were removed to a glasshouse for growing to seed, and, after first flowering, rust appeared on approximately two-thirds of the plants. This mature plant reaction to seedling inoculation prompted an investigation into the identity of bean rust races present in New Zealand, and the nature of Westralia's resistance to them.

IDENTIFICATION OF BEAN RUST RACES IN NEW ZEALAND

The lines of beans used by Fisher (1952) for differentiating rust races in the United States of America were obtained for these tests. The inoculation procedure described by Harter and Zaumeyer (1941) was carried out using collections of the New Zealand rust from the Auckland area. Their standards of symptom scoring twelve days after inoculation under glasshouse conditions were adhered to. Twelve plants of each differential line were used in each determination.

The first test was made in 1956, but three of the lines failed to germinate. Further stocks of seed were obtained, and two more tests were conducted in successive years using rust collections from the Auckland area.

The results of these three tests are given in Table 1. Comparing results with the tables of Fisher (1952), and Harter and Zaumeyer (1941) which give the reactions of differentials to 30 rust races, the possible complement in New Zealand includes races 10, 17 and 28. The combined effect of these races, however, cannot account for the consistent reading of 9 (0–10 score) on the line U.S. 780. It seems likely, therefore, that a further race unidentifiable with known races exists in this country.

TABLE 1. RUST READINGS ON DIFFERENTIAL VARIETIES INOCULATED WITH NEW ZEALAND STRAINS
(Three separate inoculations)

Differential Variety	Rust Readings (0–10 score)		
	1956	1957	1958
U.S. No. 3	—	9	9
U.S. 643	0	1	1
U.S. 650	—	10	8
U.S. 765	—	3	3
U.S. 780	9	9	9
U.S. 814	0	0	0
Golden Gate Wax	3	4	2

RESISTANCE OF WESTRALIA TO LOCAL RUST RACES

F2 Data: On testing an F2 population of the cross Westralia-Fardenlosa, 35 plants showed the same susceptible reaction as the

Fardenlosa control plants whilst 89 plants did not show rust symptoms 12 days after inoculation. The seemingly resistant plants were grown for seed, but it was noticed that 61 of these showed rust symptoms later. The remaining 28 plants did not show such symptoms throughout their growth. Thus the ratio of plants showing early, late and no symptoms of rust after seedling inoculation (35 : 61 : 28) show a good fit to a 1 : 2 : 1 ratio.

F3 Data: F3 seed from the plants showing early resistance and later susceptibility was sown in the field, and in an inoculation trial in a glasshouse. The success of natural infection in the field depends to a large extent on time of sowing. Seed sown in December-January in the Auckland area leads to general infection on susceptible varieties about one month from sowing. Because the time of infection can vary, records on F3 plants in the field trial were not taken until Fardenlosa check plants showed 100% infection.

Table 2 gives data for the rust tests (in the glasshouse and field trials) on F3 material derived from the late-symptom-showing plants selected in F2. Recordings for early rust reaction were made in the glasshouse test at 12 days from inoculation and in the field tests 44 days from sowing. Late rust reactions were recorded in the glasshouse at 22 days from inoculation and in the field 85 days from sowing.

Plants were recorded in the following classes: (1) seedling diseased, (2) seedling healthy—mature plant diseased, and (3) mature plant healthy.

The glasshouse test showed at observation in the seedling stage a reasonably good fit to a 3 healthy : 1 diseased ratio, but on later observation the reverse ratio fit occurred. Eighty-one plants first recorded as healthy became diseased, so that the numbers of plants in classes (1), (2), and (3) as previously defined were 49 : 81 : 52, a ratio which approximates a 1 : 2 : 1 ratio (see Table 2).

In the field test, an excess of healthy plants was observed at the first recording, but a greater number of diseased plants was recorded at the second. The fit to a 1 : 2 : 1 ratio is not good. Poor late growth owing to drought conditions prevented further records.

Further selections of plants of the three types from the glasshouse test gave the expected reaction on rust tests in F4, viz, (1) all progeny seedling diseased, (2) progeny segregating, (3) all progeny healthy at maturity.

F1 Data: In order to make observations on known heterozygote plants, further crosses of Westralia were made with three susceptible varieties. The reactions of F1 plants and parent varieties in a field sowing on 14 December 1955, are shown in Table 3, using the scale of infection 0–10 of Harter and Zaumeyer (1941). One month from the time rust was evident on susceptible control varieties, F1 plants gave low to intermediate readings and rust pustules were small. By March 16, however, all plants were showing strong pustule development and were scored 8–10. These reactions are as would be expected from the previous data.

TABLE 2. TWO RUST TESTS OF F3 PLANTS OF CROSS WESTRALIA × FARDENLOSA, SELECTED FOR SEEDLING RESISTANCE AND MATURE PLANT SUSCEPTIBILITY

Time of Observations	Field Test		Glasshouse Test	
	Healthy	Diseased	Healthy	Diseased
Field: 44 days from sowing	779	229		
Glasshouse: 12 days from inoculation			133	49
Field: 85 days from sowing	411	597		
Glasshouse: 22 days from inoculation			52	130

χ^2 (1 : 2 : 1) for glasshouse test 49 : 81 : 52 = 2.297
P = 0.50 —0.30

TABLE 3. RUST REACTIONS OF F1 IN CROSSES BETWEEN WESTRALIA AND SUSCEPTIBLE VARIETIES

Parentage	No. Plants	Rust Observation 26/1/56	Rust Observation 24/2/56
Westralia	20	All 0	All 0
Fardenlosa Shiny-Pod (SP)	20	All 8–9	All 10
Fardenlosa	20	All 8–9	All 10
Epicure	16	All 8–9	All 10
Fardenlosa SP × Fardenlosa	6	8, 6, 7, 8, 8, 6	All 10
Westralia × Fardenlosa SP	5	All 0	4, 2, 2, 4, 3
Fardenlosa SP × Westralia	6	All 0	4, 4, 2, 5, 4, 6
Westralia × Fardenlosa	2	All 0	2, 4
Fardenlosa × Westralia	3	All 0	4, 4, 4
Epicure × Westralia	2	All 0	5, 3

In a glasshouse trial sown on 13 November 1956, 9 plants of Westralia × Fardenlosa shiny-podded (F1) and 9 plants of Westralia × Fardenlosa (F1) were inoculated with rust on 9 December. Nine plants of each Fardenlosa parent were included as checks. Seven days after inoculation the check plants showed pustule formation, and at 12 days they were scored at 10. The crosses, however, showed no effect by the 7th day, but at 12 days small white spots appeared on leaves giving a possible score of 1. An intermediate score of 4–6 was not reached until 20 days after inoculation. At 26 days sporing was sufficiently strong to be recorded as 8–10. These results clearly indicate a delay in time of symptom expression in the heterozygotes.

Discussion

The Westralia resistance to the races of bean rust in New Zealand is most favourable for distinguishing homozygous from heterozygous individuals in segregating populations. It has been used here in the breeding programme in pedigree selection and after backcrossing.

Whether resistance or susceptibility is considered as dominant depends largely on the interpretation of the response to rust of the heterozygote. The phenotypic expression of the heterozygote can be described as a delayed susceptibility or a resistance at an early growth stage. An identical situation in immunity of peas to pea mosaic virus led to susceptibility being named dominant (Yen and Fry 1956).

The condition recalls the "intermediate" description applied to the heterozygotes in F2 populations by Zaumeyer and Harter (1941) working with one of the races (Race 17) which has been found in the complex here. In that case, heterozygotes could be scored as intermediates in a population in terms of a system of estimation of pustule size *at the same time* as susceptible parent controls showed the maximum or near-maximum scores. In the present material, and in all the breeding material that has been tested, no single case of this could be recorded without a distinct time lag. Zaumeyer and Harter tested the progeny of F2 plants scored as intermediates, and in one case recorded a 3 : 1 resistant : susceptible ratio. The development of this rather than the 1 : 2 : 1 ratio was attributed to "a lack of the proper environment for the maximum development of rust fungus". The authors pointed out that their work referred only to seedling reactions.

Many authors have recorded seedling reaction to disease differing from adult plant reaction in hybrid material (summarised by Zaumeyer and Harter 1941; Vavilov 1950), particularly with cereal rust. Goulden *et al.* (1928), on the basis of inoculations at different growth stages, concluded that juvenile and adult resistance to wheat stem rust were controlled independently. There is no evidence of two resistance systems in this case where, in controlled tests, reactions were the result of a single inoculation. In the outdoor tests, where infection was natural, heterozygotes still showed a delayed but positive reaction to the disease.

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VITAMIN B_{12} AND COBALT IN LIVERS FROM GRAZING COBALT-DEFICIENT LAMBS AND FROM OTHERS GIVEN VARIOUS COBALT SUPPLEMENTS

By E. D. Andrews, L. I. Hart, and B. J. Stephenson, Animal Research Station, Department of Agriculture, Wallaceville

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Summary

Vitamin B_{12} and cobalt concentrations were determined in the livers of 4 groups of lambs. Three groups were grazed on cobalt-deficient pastures. Of these groups one received no cobalt treatment and developed marked cobalt deficiency disease. Of the other two groups on cobalt-deficient pastures, one received oral doses of cobalt and the other cobaltic oxide pellets. The fourth group was grazed on cobalt-topdressed pastures.

Vitamin B_{12} concentrations in the livers of markedly cobalt-deficient lambs ranged from 0.026 to 0.097 μg/g of wet tissue, with a mean of 0.062 μg/g. Cobalt dosing and cobaltic oxide pellets increased liver vitamin B_{12} concentrations about fourfold and sevenfold respectively. Vitamin B_{12} concentrations in the livers of animals from cobalt-topdressed pasture were of an order similar to those for lambs given cobaltic oxide pellets.

Effects of cobalt deficiency disease and of cobalt treatments on B_{12} Co/total Co ratios are discussed.

From all the information available it is concluded that vitamin B_{12} concentrations should provide a more sensitive indication of the presence or absence of cobalt deficiency disease than do cobalt concentrations. For sheep a value of 0.20 μg/g is suggested as an approximation to the minimal liver vitamin B_{12} concentration ("critical level") consistently associated with freedom from cobalt deficiency disease. Tentative vitamin B_{12} diagnostic criteria are given.

Introduction

Underwood (1956) has reviewed the work which, first in Australia, and later in New Zealand and elsewhere, showed that in certain localities wasting diseases of cattle and sheep were due to a deficiency of cobalt. These diseases could be cured and prevented by increasing the oral cobalt intake of animals grazing on the deficient areas. Since that time work carried out in Australasia and elsewhere has resulted in an extensive literature on the cobalt content of liver. In particular, liver analyses have proved a valuable, though not infallible diagnostic aid to the detection of cobalt deficiency in practice (McNaught 1948), and for the past 14 years have been used continuously for this purpose at Wallaceville (Andrews, Stephenson, Anderson, and Faithful 1958).

More recently a number of workers have shown that cobalt deficiency disease cannot be controlled by injections of inorganic cobalt. Small amounts of injected vitamin B_{12} are, however, effective. Johnson and Bentley (1958) have reviewed evidence which shows that vitamin B_{12} is synthesised from cobalt by micro-organisms in the rumen; in cobalt-deficient animals vitamin B_{12} production is reduced. Further-

more, the present writers have found no evidence from the literature that, in animals, cobalt exerts a normal physiological effect in any form other than vitamin B_{12}. These facts indicate that cobalt deficiency disease is, in effect, due to a deficiency of vitamin B_{12}.

Under certain special experimental conditions cobalt concentrations in liver do not provide a measure of the vitamin B_{12} status of ruminants. Thus injections of inorganic cobalt will raise the liver cobalt to levels many times greater than those found for normal animals (Marston and Lee 1949; Becker *et al.* 1949; Phillipson and Mitchell 1952), but since these injections will not control cobalt deficiency disease this store is apparently not available in significant amounts for the synthesis of vitamin B_{12} in the rumen. On the other hand Andrews (unpublished results quoted by Underwood 1956) found that vitamin B_{12} injections in amounts sufficient to maintain lambs on cobalt-deficient land in a healthy condition failed to raise the cobalt contents of their livers above the low levels known to be associated with cobalt deficiency disease in the field. To quote Underwood (1956) "It is apparent that under the appropriate conditions freedom from signs of cobalt deficiency disease can be compatible with a low liver cobalt status, just as cobalt deficiency symptoms can be compatible with a high liver cobalt status". Thus it appears that in practice the reliability of liver cobalt determinations for diagnostic use will depend upon the extent to which they reflect the vitamin B_{12} status of the animal. A measure of the variability between animals in the proportion of presumably "active" (B_{12}) cobalt to "active plus inactive" (total) cobalt should give an indication of the reliability of total cobalt concentrations.

To examine this particular point, and the more general one that vitamin B_{12} determinations should give a direct approach to the laboratory diagnosis of cobalt deficiency disease, we have compared the vitamin B_{12} and cobalt concentrations in the livers of cobalt-deficient lambs and healthy lambs including groups given extra cobalt. Part of this work has been reported on briefly (Andrews, Hart, and Stephenson 1958). In another paper (Andrews *et al.* 1959) we reported more fully on the vitamin B_{12} and cobalt concentrations in livers from normal unsupplemented lambs, cobalt dosed lambs, and lambs with a recent history of mild cobalt deficiency disease. Later, in a field trial, the effect of cobaltic oxide pellets on the growth of lambs grazing cobalt-deficient pastures was investigated (Andrews, Isaacs, and Findlay 1958). This experiment afforded us an opportunity of examining the vitamin B_{12} and cobalt levels in the livers of markedly cobalt-deficient lambs, lambs given oral doses of cobalt sulphate, lambs given cobaltic oxide pellets, and a fourth group grazing pastures topdressed with cobalt sulphate. These results are reported in this paper. From this and other information, diagnostic criteria based on vitamin B_{12} concentrations in liver have been devised and are presented here.

EXPERIMENTAL

Field Trial

Two paddocks, Nos. 7 and 8A, at the Winton Experimental Farm, Southland, were used. Paddock 7 had produced cobalt-deficient lambs

in previous years. Paddock 8A, which adjoined paddock 7, was of the same soil type (Otapiri silt loam) and was of similar pasture composition. It was topdressed with cobalt sulphate at the standard annual rate of 5 oz per acre in November 1956. Pasture samples for cobalt analyses were taken from paddock 7 in December 1956, and from paddocks 7 and 8A in January and April 1957.

Four groups of lambs, born about September 1956, were available.

Group 1.	Control	(22 lambs)
2.	Cobalt sulphate dosed, 7 mg Co/lamb/week	(12 lambs)
3.	Cobaltic oxide pellets, one each lamb	(12 lambs)
4.	On cobalt sulphate topdressed pasture	(10 lambs)

Details of Groups 1, 2 and 3, which were run together from birth and throughout the trial period on the known cobalt-deficient paddock (No. 7), are given in a previous publication (Andrews, Isaacs and Findlay 1958). The pellets given to Group 3 lambs were from an early experimental batch made available to us by CSIRO, Division of Biochemistry and General Nutrition, Adelaide, South Australia, and contained 75% black cobaltic oxide. Lambs in Group 4 were set stocked from birth on the adjoining cobalt-topdressed paddock (No. 8A). Experimental treatments of the lambs in Groups 1, 2, and 3 commenced on 24 January 1957, when the mean weight of the lambs was 64.7 lb. On the other hand the mean weight of the lambs in the cobalt-topdressed paddock was 71.4 lb at this time. From this, and from experience of the time of onset of cobalt deficiency symptoms in previous years, it was considered likely that lambs in paddock 7 (Groups 1, 2 and 3) were already affected by the disease when treatments commenced.

Throughout the trial period lambs in paddock 7 were weighed at weekly intervals and those in paddock 8A at fortnightly intervals. After 14 weeks (on 1 May 1957) all lambs were killed at the freezing works. The livers were collected, weighed fresh, frozen, and air-freighted in vacuum flasks to the Wallaceville Animal Research Station.

Laboratory Procedures

Cobalt concentrations in pastures and livers and vitamin B_{12} concentrations in livers were determined by the methods previously used (Andrews *et al.* 1959). The vitamin B_{12} assay organism used (*Lactobacillus leichmannii* ATCC 7830) will respond also to some vitamin B_{12}-like factors inactive for higher animals (Ford and Hutner 1955). However, this is of no consequence in the present work, as identical results have been obtained when ovine liver is assayed with both *L. leichmannii* ATCC 7830 and with the B_{12}-specific organism *Ochromonas malhamensis* (Hine and Dawbarn 1954) showing that the vitamin B_{12} activity in ovine liver consists essentially of one or both of the animal-active forms cyano- and hydroxo-cobalamin. A similar conclusion has been reached for bovine liver (Ford *et al.* 1953).

Vitamin B_{12} concentrations are expressed as μg/g on a wet-weight (w.w.) basis and cobalt concentrations as μg/g on a dry-weight (d.w.) basis. To relate vitamin B_{12} and cobalt figures on a common basis,

dry-matter determinations were carried out on all livers. Total vitamin B_{12} contents of livers were calculated from the corresponding concentrations and wet weights of whole livers, and total cobalt contents from the corresponding concentrations and dry weights of whole livers. Vitamin B_{12}-cobalt/total cobalt ratios for all livers were estimated from the vitamin B_{12} concentrations (converted to a dry-weight basis), the cobalt concentrations, and the cobalt content of the vitamin (4.3%) as determined by its formula.

Statistical Analyses

Statistical examination of results for Groups 1, 2 and 3 were carried out by Mr T. G. Robertson, Department of Agriculture, Wellington. Results for Group 4 have not been included in the statistical analyses. As previously mentioned, lambs in Groups 1, 2, and 3 were run together on the same paddock, whereas those in Group 4 were run on the separate cobalt-topdressed paddock. Since the trial could not be replicated on a paddock basis, any effects due to paddock differences other than those caused by cobalt topdressing could produce a bias in the comparisons made. For this reason comparison of results for Group 4 with those for the other three groups are made in general terms only.

RESULTS

Cobalt Concentrations in Pastures and Lamb Weight Changes

Cobalt concentrations in pasture samples are shown in Table 1.

TABLE 1. COBALT CONCENTRATIONS (μg/g d.w.) IN PASTURE SAMPLES

Paddock No.	Dates Sampled		
	4/12/56	24/1/57	30/4/57
7	0.08	0.06	0.05
8A	—	0.27	0.07

The values for paddock 7 are consistent with cobalt deficiency disease in lambs (Andrews, Stephenson, Anderson, and Faithful 1958). The figure for the January sample from the cobalt-topdressed paddock (8A) is well above deficiency levels, but by the end of April the cobalt in pasture from this paddock had fallen to a low value consistent with cobalt deficiency disease.

During the trial period 7 lambs in the control group died after losing weight in amounts ranging from 4 to 21 lb. From three of these (No. 7134, died 5 March; Nos. 7142 and 7120, died 13 March) fresh livers were obtained for vitamin B_{12} and cobalt determinations (see Table 2). There were no deaths in the other 3 groups. At the end of the trial the mean live-weight for surviving control lambs (Group 1) was 61.2 lb. The mean live-weights for Groups 2, 3 and 4 were

similar; 87.2, 88.2 and 87.5 lb respectively. At slaughter cobaltic oxide pellets were not found in two lambs from Group 2. One other pellet was heavily encrusted with calcium phosphate.

Mean weight changes at each weighing for Groups 1, 2, and 3 are given elsewhere (Andrews, Isaacs, and Findlay 1958).

Weight changes for each lamb in all 4 groups, between 24 January and 30 April, are given in Table 2. Differences between means for Groups 1 and 2, and between groups 1 and 3 are statistically highly significant. There is no significant difference between means for Groups 2 and 3. The mean gain shown for Group 4 is discussed later.

Dry Matter Content of Livers

These figures are of incidental interest only and are not given in detail here. Mean values as percentages, for Groups 1, 2, 3, and 4, were 27.8, 28.8, 28.7 and 35.0 respectively. On their appearance when dried for cobalt analyses, two livers from the control group (lambs 7120 and 7138, Table 2) were classified as "very fatty". The dry matter content of these two livers proved abnormally high, 41.5% and 44.0% respectively. The mean dry matter content of livers from Group 4 (paddock 8A) was considerably higher than the mean dry matter contents of livers from the other 3 groups. The reason for this is not known.

Vitamin B_{12} and Cobalt in Livers

In the light of present knowledge, total vitamin B_{12} and total cobalt contents of livers from individual animals convey to the present writers no greater physiological meaning than corresponding results expressed as concentrations. However, we consider it prudent to record here the total values expressed as means for each group. Mean total vitamin B_{12} contents (μg) for Groups 1, 2, 3 and 4 were respectively 31, 176, 285 and 281. Mean cobalt contents (μg) were respectively 4, 40, 21 and 28.

Detailed results for vitamin B_{12} concentrations, cobalt concentrations and B_{12} Co/total Co ratios are shown in Table 2. For ease of comparison vitamin B_{12} concentrations within each group are arranged in descending order of magnitude.

Within the control group (1) the correlation between weight changes and vitamin B_{12} concentrations for lambs surviving to the end of the experiment is statistically highly significant ($r = 0.65$). In this group there is no significant correlation between weight changes and cobalt concentrations. Correlations between weight changes and vitamin B_{12} concentrations, and between weight changes and cobalt concentrations are not significant within the other 3 groups. Only within Group 1 is there significant correlation between weight gains and B_{12} Co/total Co ratios. There are no significant correlations between vitamin B_{12} concentrations and cobalt concentrations within any of the 4 groups.

Table 2 shows that mean vitamin B_{12} concentrations for Group 1 lambs range downward from about 0.10 μg/g with a mean of 0.062

TABLE 2. VITAMIN B_{12} AND COBALT CONCENTRATIONS IN LIVERS OF COBALT-DEFICIENT AND COBALT-SUPPLEMENTED LAMBS

Group			Lamb No.	Weight Change (lb)	Liver Determinations		
No.	Paddock	Treatment			Vitamin B_{12} (μg/g w.w.)	Cobalt (μg/g d.w.)	B_{12}Co Total Co
1	7	None (Control)	7106	— 5	0.097	0.03	0.5
			7115	— 5	0.084	0.06	0.2
			7112	4	0.081	0.02	0.6
			7101	—10	0.080	0.03	0.5
			7105	8	0.079	0.03	0.4
			7103	5	0.072	0.02	0.6
			7114	— 6	0.071	0.03	0.5
			7127	— 9	0.062	0.03	0.3
			7121	— 8	0.060	0.03	0.3
			7134*	— 9	0.053	0.06	0.2
			7122	— 5	0.048	0.05	0.1
			7142*	—10	0.047	0.08	0.1
			7136	0	0.046	0.02	0.4
			7194	—16	0.045	0.02	0.4
			7141	—12	0.041	0.03	0.2
			7108	—16	0.036	0.02	0.2
			7120*	—15	0.033	0.03	0.1
			7138	—27	0.026	0.02	0.1
			Means	— 6.8	0.0619	0.029	0.35
2	7	Cobalt sulphate doses (7mg Co/ lamb/week)	7131	30	0.51	0.22	0.35
			7119	14	0.40	0.24	0.24
			7132	26	0.38	0.29	0.20
			7133	27	0.34	0.18	0.29
			7124	24	0.26	0.19	0.20
			7129	36	0.24	0.22	0.17
			7117	18	0.22	0.13	0.25
			7111	26	0.21	0.24	0.12
			7118	23	0.19	0.18	0.16
			7130	27	0.18	0.32	0.08
			7126	5	0.17	0.35	0.08
			7128	22	0.15	0.13	0.17
			Means	23.2	0.271	0.224	0.193
3	7	Cobaltic oxide pellets	7123	23	0.76	0.13	0.88
			7140	30	0.64	0.17	0.55
			7104	24	0.54	0.12	0.68
			7107**	25	0.47	0.12	0.56
			7102†	25	0.47	0.11	0.63
			7145	27	0.45	0.10	0.68
			7143	24	0.43	0.11	0.57
			7125	22	0.41	0.12	0.52
			7135	29	0.35	0.08	0.65
			7109	11	0.31	0.13	0.36
			7116**	29	0.30	0.08	0.55
			7139	24	0.27	0.11	0.38
			Means	24.4	0.450	0.116	0.584
4	8A	Cobalt sulphate top-dressed paddock (5 oz/ acre)	7150	13	0.81	0.16	0.66
			7149	14	0.75	0.16	0.56
			7148	22	0.74	0.18	0.52
			7154	18	0.58	0.12	0.56
			7156	15	0.55	0.15	0.56
			7147	16	0.53	0.13	0.50
			7153	20	0.47	0.17	0.35
			7152	21	0.32	0.10	0.42
			7151	8	0.24	0.17	0.17
			7155	14	0.20	0.15	0.15
			Means	16.1	0.519	0.146	0.445

* Died before end of trial: results not included in means.
** Pellets not found.
† Pellet heavily coated with calcium phosphate.

μg/g. Statistical analyses showed that the mean vitamin B_{12} concentrations for Groups 2 and 3 are highly significantly greater than the mean for Group 1. The difference between means for Groups 2 and 3 in favour of the latter is also highly significant. In Group 3, vitamin B_{12} figures for livers from lambs from which pellets were not recovered (Nos. 7107 and 7116) and for the liver from the lamb with the heavily coated pellet (No. 7102) fall within the range of other values for this group. The range and mean of vitamin B_{12} concentrations for livers from lambs in the cobalt-topdressed paddock (Group 4) are broadly similar to corresponding results for livers from lambs given cobalt pellets (Group 3).

For mean cobalt concentrations and mean B_{12} Co/total Co ratios there were in all cases highly significant differences among Groups 1, 2, and 3 taken in pairs.

It is evident that cobalt concentrations and B_{12} Co/total Co ratios for Group 4 are of a similar order to those found for Group 3.

Discussion

Effects of Cobalt Treatments on Lamb Growth Rates

From a comparison of results for Groups 2 and 3 with those for Group 1 it is evident that, as a group, control animals were markedly cobalt-deficient. Furthermore, individual weight changes suggest that all animals in the control group were probably affected by the disease to a greater or lesser degree.

The low weight gain made by lamb 7126 in Group 2 is obviously not typical of the gains for the rest of the lambs in this group. A post-mortem examination of this lamb made by Mr C. E. Isaacs, Government Veterinarian, Invercargill, revealed no obvious abnormalities, but since 7 mg of cobalt each week will maintain sheep free from cobalt deficiency (Lee 1950) it is presumed that the failure to grow normally was due to some cause other than this disease. Since the mean gain made by Group 3 is comparable to that for Group 2 it appears that cobaltic oxide pellets provided sufficient cobalt to maintain lambs free from cobalt deficiency disease under the conditions of this trial.

The mean weight gain for lambs in Group 4 (Paddock 8A) was appreciably less than the mean weight gains for lambs in Groups 2 and 3 (paddock 7), and this requires comment. Although pasture cobalt had fallen to a low level in paddock 8A by the end of the trial, results from many field experiments carried out elsewhere in New Zealand (N.Z. Dept. Agric. 1954) have shown that annual applications of 5 oz of cobalt sulphate per acre will keep sheep continuously free from cobalt deficiency disease. In particular, a previous trial carried out at the Winton Experimental Farm on the same paddocks as those used in the present trial showed that prior grazing of cobalt-topdressed pastures for 3 months protected lambs transferred to cobalt-deficient land against the disease for at least a further 4 months (Andrews and Anderson 1955). In the present trial all groups lost weight after

dipping. Otherwise, successive weighings showed that lambs in Group 4 grew at a steady rate throughout the period. Moreover, as discussed later, liver vitamin B_{12} stores for this group were adequate. For these reasons it is concluded that lambs in Group 4 (paddock 8A) were free from cobalt deficiency at the end of the trial. Thus, no definite reason can be given for their lower mean weight gain as compared with cobalt-treated lambs in Groups 2 and 3 (paddock 7). Possibly there was some difference between paddocks in nutritive value or palatability of pastures. Alternatively, since lambs in paddock 7 were probably cobalt-deficient at the start of the trial, those given cobalt supplements thereafter may have grown at a faster rate than they would otherwise have done had their growth not been interrupted.

Effect of Cobalt Treatments on Vitamin B_{12} Concentrations

Under the conditions of the present experiment, weekly cobalt dosing on an average increased the vitamin B_{12} concentration in the liver about fourfold. Cobaltic oxide pellets were on an average more effective than cobalt dosing in increasing vitamin B_{12} concentration and caused an approximate sevenfold increase. These pellets, however, contained 75% cobaltic oxide. The pellet now available commercially contains 90% cobaltic oxide and might have given an appreciably different result. However, results from subsequent unpublished experiments suggest that the commercial pellet is at least no less effective in raising liver vitamin B_{12} levels.

Although they were not recovered, the pellets given to lambs 7107 and 7116 were effective in promoting growth and raising vitamin B_{12} concentrations. The pellet given to lamb 7102 and which became heavily encrusted with calcium phosphate was similarly effective. But in a later trial with unweaned lambs (Andrews 1959) one animal in the pelleted group died in very poor condition The pellet was recovered and found to be heavily encrusted. The vitamin B_{12} concentration (0.068 μg/g) was of similar order to those found for cobalt-deficient lambs in the present trial, indicating that in some circumstances calcium phosphate coating may render pellets ineffective.

Vitamin B_{12} concentrations in livers from lambs in the cobalt-topdressed paddock (Group 4) were similar in range and mean to those found for lambs given pellets, and were therefore adequate. Since the trial paddocks were not replicated, there is, of course, no means of establishing precisely the extent to which topdressing caused an increase in the vitamin B_{12} content of livers. However, there were no marked visible differences between cobalt-topdressed and non-topdressed experimental pastures and the paddocks are of the same soil type. Analysis of the pasture sample taken from the topdressed paddock at the end of the trial showed a low cobalt content. From these considerations we think it likely that the amount of naturally-occurring cobalt from the topdressed pasture was probably low throughout the trial and that had topdressing not been carried out the animals would have developed cobalt deficiency disease at least to a mild degree. If this were true it follows from previous work (Andrews *et al.* 1959) that vitamin B_{12} concentrations found in the livers were largely due to the effect of cobalt topdressing.

Comparisons of B_{12} Co/Total Co Ratios

Effect of Cobalt Deficiency. The mean ratio for cobalt-deficient lambs is less than half that previously found (Andrews *et al.* 1959) for lambs grazing pastures containing sufficient naturally occurring cobalt. However, the low cobalt concentrations found in the livers of cobalt-deficient animals approach the limits of sensitivity of the spectrophotometric analytical method we used. Consequently, any small constant error which tended to over-estimate the amount of cobalt present would be proportionately large at low concentrations and so account, at least in part, for the results obtained. It is not known whether such an error exists and a more sensitive analytical technique is being sought at Wallaceville to examine this point. Meanwhile, our findings are supported by a few similar results from British investigations (Dr A. T. Phillipson, pers. comm.) since in the latter, cobalt concentrations were measured spectrographically.

If the phenomenon is a real one it might be explained on the grounds that cobalt deficiency results in a more rapid mobilisation of vitamin B_{12} than of non-B_{12} Co from the liver. Alternatively, cobalt deficnency may result in impaired absorption of vitamin B_{12} as compared with non-B_{12} Co. On the other hand, virtually none of the cobalt in the livers of at least some lambs grazing pastures containing sufficient naturally-occurring cobalt appears to be in a non-B_{12} form (Andrews *et al.* 1959). A better explanation may be that in cobalt-deficient animals a proportionately greater amount of ingested cobalt fails to be converted to vitamin B_{12} in the rumen, and this is reflected in a relatively increased non-B_{12} Co concentration in the liver.

Effects of Cobalt Treatments. It was previously reported (Andrews *et al.* 1959) that B_{12} Co/total Co ratios for lambs grazing pastures containing sufficient naturally-occurring cobalt ranged from 0.7 to 1.0 with a mean of 0.8. A comparison of these figures with those for lambs in Groups 3 and 4 suggests that the proportion of non-B_{12} Co in the liver may be higher when inorganic cobalt supplements are provided either as pellets or as topdressing. In the previous work it was found that most of the cobalt in the livers of lambs given weekly oral doses of cobalt tended to be in some form other than vitamin B_{12}. Results for Group 2 in the present experiment substantiate this finding.

Diagnostic Criteria

It is generally accepted that the most certain method of establishing the presence or absence of cobalt deficiency disease is by a controlled cobalt supplementation trial in the field. After a suitable period, which may be of the order of 3 months, the mean weight changes of control and cobalt-treated groups are compared and a diagnosis made on the results obtained. In practice this method may have some disadvantages. It is not always possible to conduct the most efficient kind of trial because it may interfere with normal farm routine. Under some circumstances field trials may not, in fact, be practicable. Also, the time required to reach a diagnosis is considerable. This could result in appreciable economic loss to the farmer. For these reasons results from laboratory examinations of liver samples

are a useful means of assisting in the diagnosis of cobalt deficiency disease.

Of necessity only one or two livers from each farm can be subjected to laboratory examination. These may not necessarily be representative of those from the whole flock. It can also be expected that whatever the laboratory method employed, borderline values will always present difficulties of interpretation in terms of the presence or absence of cobalt deficiency disease. Cobalt and vitamin B_{12} diagnostic criteria are discussed here subject to these reservations.

Cobalt. Criteria, based on the work of McNaught (1948), and routinely used at Wallaceville, have been given elsewhere (Andrews, Stephenson, Anderson, and Faithful 1958). In terms of these, except for one borderline value, all of the livers from the cobalt-deficient group would be classified as low. Cobalt figures for all lambs in the orally dosed group are well above accepted deficiency levels. But from the low B_{12} Co/total Co ratios found for this group it is evident that, as an indication of the vitamin B_{12} status of the animal, cobalt figures would be misleading. For lambs treated in this way it is conceivable that if the treatment were withdrawn, cobalt deficiency, with concomitant low vitamin B_{12} values, could eventually supervene, despite relatively high residual cobalt in the livers. A similar criticism may, in lesser degree, apply for other forms of cobalt supplementation. However, in the present work borderline values were found for two lambs from the group given cobalt oxide pellets. Had one or both of these particular livers been selected by chance this would have raised the possibility of cobalt deficiency when, in fact, no apparent de-deficiency existed. With the reservation noted above for cobalt-treated animals generally, other values for this group and for the group on cobalt-topdressed pasture indicated correctly that no cobalt deficiency was present.

If it is accepted that for practical purposes vitamin B_{12} is the only physiologically active form in which cobalt occurs in animal tissues, a general criticism of criteria based on cobalt analyses arises from consideration of B_{12} Co/total Co ratios. It is evident that these ratios can vary greatly within groups apparently subjected to the same environment. It follows that cobalt figures reflect vitamin B_{12} levels only within wide limits and that their value in assisting in the diagnosis of cobalt deficiency disease is correspondingly limited.

Vitamin B_{12}. Present results suggest that vitamin B_{12} concentrations in the livers of markedly cobalt-deficient lambs are invariably lower than concentrations in the livers of those receiving sufficient cobalt. Moreover, the findings demonstrate that within a group of cobalt-deficient lambs, vitamin B_{12} concentrations, in contrast to cobalt concentrations, tend to be related to the degree of deficiency of individuals as measured by weight changes. For these reasons, and because of the variability in B_{12} Co/total Co ratios discussed above, we are of the opinion that vitamin B_{12} concentrations should provide a more sensitive indication of the presence or absence of cobalt deficiency disease than do cobalt concentrations.

In the discussion that follows we have generalised from present results and other information in an attempt to provide vitamin B_{12} diagnostic criteria for sheep. For this purpose the "critical level" of vitamin B_{12} in livers of sheep is defined as the minimal concentration consistently associated with freedom from cobalt deficiency disease. No attempt is made to differentiate between a critical level for lambs and for older sheep.

Previously we reported (Andrews, Hart, and Stephenson 1958) that vitamin B_{12} concentrations in the livers of 10 lambs, moribund or dead from cobalt deficiency, ranged downward in all cases from 0.068 μg/g. Figures for the 3 control lambs that died during the present trial were included among these. Thus, concentrations of 0.07 μg/g or less are considered as indicative of acute cobalt deficiency disease.

Vitamin B_{12} concentrations in the livers of our 15 control lambs that survived to the end of the trial ranged from 0.026 to 0.097 μg/g. Other unpublished results from controlled trials carried out elsewhere have shown that values for livers from markedly cobalt-deficient ewes and lambs have invariably fallen within or below this range. American workers (Hoekstra *et al.* 1952; Kercher and Smith 1956) have reported vitamin B_{12} results for livers from cobalt-deficient lambs on dry rations. Australian figures are available for similarly fed, cobalt-deficient sheep of unstated ages (Dawbarn *et al.* 1958). These figures, in all cases, fall within the range of values given above. Thus it appears that for sheep generally liver vitamin B_{12} concentrations of 0.10 μg/g or less are indicative of definite cobalt deficiency disease.

From a previous report (Andrews *et al.* 1959) it is considered that cobalt deficiency is not likely to be present in lambs with liver vitamin B_{12} concentrations greater than 0.30 μg/g. However, a rather lower value for the critical level is suggested by present findings for cobalt-treated lambs and by other results from the previous trial. These latter indicated that vitamin B_{12} concentrations ranging downward from an order of 0.30 μg/g to one of 0.20 μg/g could be consistent with recovery from recent mild cobalt deficiency. In another controlled trial (unpublished), we found that vitamin B_{12} concentrations in the livers of two sheep from a group that was mildly cobalt-deficient as judged by reduced weight gains, were 0.11 and 0.15 μg/g. Australian work (Dawbarn *et al.* 1958) suggests that biochemical lesions may result when vitamin B_{12} concentrations drop to values intermediate between 0.14 and 0.19 μg/g since folic acid and citrovorum factor concentrations were normal at the latter level but were reduced several fold at the former and lower levels. In the present work vitamin B_{12} concentrations in livers from cobalt-supplemented lambs not evidently affected by cobalt deficiency ranged down to 0.15 μg/g. However, it is common knowledge that within a group of animals apparently continuously subjected to the same environmental influences, individuals differ, not only in susceptibility to cobalt deficiency, but in various other ways. It is not known to what extent these differences influence vitamin B_{12} storage in the liver, but it can be accepted that they will set a limit to the precision with which vitamin B_{12} levels in livers from individual lambs can be related to the presence or absence of cobalt

deficiency. In particular it may not be invariably true that individuals with liver vitamin B_{12} values between 0.15 and 0.19 μg/g inclusively are necessarily free from the disease. From the foregoing we consider that for practical purposes an approximation to the critical level can be tentatively set at 0.20 μg/g.

In considering values ranging between 0.11 and 0.19 μg/g inclusively, which we may term the "borderline range", it is reasonable to suppose that vitamin B_{12} figures for livers from mildly affected sheep will tend to approach the lower value, and figures for livers from animals whose cobalt requirements have barely been met will tend to be nearer the higher or critical level. However, figures within the borderline range are obviously of less certain interpretation than figures within either higher or lower ranges, and therefore are of more limited diagnostic value. For example, vitamin B_{12} results for 3 of the 34 cobalt-treated lambs in the present trial would have raised the possibility of cobalt deficiency, although presumably no deficiency was present. Experiments designed to set the limits of the borderline range more precisely are in progress.

The foregoing discussion is summarised in the following vitamin B_{12} criteria:

Very low	< 0.07 μg/g
Low	0.07–0.10 μg/g
Borderline	0.11–0.19 μg/g
Normal	> 0.19 μg/g

These criteria may be subject to future amendment. Meanwhile, they have been adopted at Wallaceville for routine use.

Recent Australian work (Skerman *et al.* 1959) suggests that criteria of a similar order apply also for cattle.

Acknowledgments

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MAGNESIUM LOSSES FROM HOROTIU SANDY LOAM FOLLOWING APPLICATION OF POTASSIUM CHLORIDE

By D. E. Hogg, Rukuhia Soil Research Station, Department of Agriculture, Hamilton

(*Received for publication, 6 January 1960*)

Summary

Laboratory and field investigations on Horotiu sandy loam indicate a loss of magnesium as a result of KCl topdressing. Losses are increased by the addition of nitrogenous fertilisers. KCl applications cause a greater initial loss of magnesium than do K_2SO_4 applications.

Introduction

A decrease in exchangeable magnesium levels is commonly observed following topdressing of pastures. In a survey of fertilised and unfertilised Waikato soils, Dixon and Taylor (1942) attributed the decrease to the action of the calcium sulphate present in superphosphate, with probable loss of Mg in the drainage water. Boynton and Burrell (1944) found a very significant decrease in exchangeable Mg in surface soil after 5 years of potassium fertilisation. Cooper *et al.* (1934) observed that magnesium deficiency in cotton was usually most prevalent when the rainfall during the growing season was relatively heavy. They attributed this to the formation of soluble magnesium salts following the application of sulphates, chlorides, and nitrates in fertilisers, with consequent leaching of Mg from the soil. In view of the greatly increased use of potassium in New Zealand, and the trend towards the use of nitrogenous fertilisers for out-of-season production, it was considered advisable to investigate the effects of potassium and nitrogen applications on soil magnesium levels.

Experimental

It was decided to apply fertiliser, in solid form, to the surface of columns of soil and leach with distilled water, periodically examining the leachate for bases removed. As previous experience had shown that columns of soil in the laboratory failed to drain after saturation, but remained water-logged for periods up to a fortnight, some method of partial drying of the soil after saturation was necessary. This was achieved by conducting the experiment in a temperature-controlled room (25°c) and by placing the soils directly in front of a fan after each leaching. With this technique, a cycle of wetting and drying was obtained which is similar to that occurring in the field.

The soil used was Horotiu sandy loam, obtained from a 0–6 in. depth. Ammonium acetate leaching gave the following data.

		Exchangeable Cations (me.%)				
C.E.C.	T.E.B.	Ca	Mg	K	Na	Satn. (%)
24.6	12.1	10.6	0.73	0.20	0.58	49

The soil container consisted of a polythene bottle, $2\frac{1}{4}$ in. diameter, 4 in. deep, with the bottom removed. This was inverted, and a filter disc, covered with a circle of filter paper, placed in the neck. Sufficient soil (175 g, air-dried and passed 2 mm sieve) was placed in the container to bring the level to within $\frac{1}{4}$ in. of the top. The soil was then set on a stand, so that any leachate passing through collected in a 50 ml Kolrausch flask placed directly underneath.

Treatments

(1) Control
(2) 0.130 g KCl (1 me.% K).
(3) 0.130 g KCl + 0.115 g $(NH_4)_2SO_4$ (1 me.% N).
(4) 0.130 g KCl + 0.115 g nitro-lime (approx. 1 me.% N).
(5) 0.152 g K_2SO_4 (1 me.% K).

To simulate normal field practice, all soils, including control, received a prior application of 0.167 g of superphosphate (approx. equivalent to 4 cwt per acre) placed on the surface and lightly worked in. The treatments, which were carried out in duplicate, were then added in solid form and mixed in to the top $\frac{1}{4}$ in. of soil. Distilled water was added in small amounts (5–10 ml) over a period of approximately 8 hours, until 50 ml of leachate had collected in the Kolrausch flask. The containers were then removed from the stand and placed directly in front of a fan, for a period of 60 hours. This technique conveniently allowed two leachings per week, with one drying period in the middle of the week, and the other over the weekend. To reduce the number of analyses, the two leachates from each week were added together and the cations determined in the one solution. Na, K, and Ca were determined by flame photometry and Mg by atomic absorption (Allan 1958). With the exception of the first application of water (when the soil was air dry), approximately $2\frac{3}{4}$ in. of water had to be applied in order to collect 1 in. in the drainage.

Results

Cation contents of the drainage water are set out in Table 1.

Magnesium

(a) The most striking point emerging from the data presented, lies in the immediate release of Mg following the application of potassium chloride. Losses dropped steeply after the first week.

(b) The amount of magnesium leached out was increased by the addition of ammonium sulphate or nitro-lime (Fig. 1).

(c) When potassium was applied as K_2SO_4, release of Mg was more gradual (Fig. 2), although the total amount lost over the eight-week period was not markedly different (0.25 me.% for KCl, 0.21 me. for K_2SO_4).

Calcium and Sodium

The pattern of release was similar to that of magnesium, showing a greater early loss from the KCl treatment than from the K_2SO_4 application.

TABLE 1. CATION CONTENT OF DRAINAGE WATER, FOLLOWING FERTILISER APPLICATION ON HOROTIU SANDY LOAM

Treatment	Week	Cations Leached			
		Mg me.%	Ca me.%	Na me.%	K me.%
(1) Control	1	.018	.152	.055	.004
	2	.018	.091	.049	.002
	3	.020	.104	028	.002
	4	.017	.091	.018	.002
	5	.012	.073	.011	.001
	6	.011	.057	.008	001
	7	.008	.049	.006	nil
	8	.007	.040	.005	nil
(2) KCl	1	.155	.774	.125	.016
	2	.034	.162	044	.025
	3	.020	.098	.022	.034
	4	.016	.082	.014	.037
	5	.011	.063	.008	.033
	6	.007	.043	.006	.031
	7	.006	.035	.005	028
	8	.005	.028	003	.026
(3) KCl + $(NH_4)_2SO_4$	1	.194	.855	.143	.041
	2	.075	.387	049	.086
	3	.061	.382	.020	.088
	4	.023	.134	.009	.060
	5	.015	.096	.006	.044
	6	.010	.070	.005	035
	7	.009	059	.004	.031
	8	.007	.052	.003	.028
(4) KCl + nitro-lime	1	.237	1.194	.149	029
	2	.056	.288	.046	.074
	3	.021	.125	.016	.065
	4	012	.066	.008	.052
	5	.008	.053	.006	.042
	6	005	.039	.005	.034
	7	.005	.033	.004	.030
	8	.005	.027	003	.027
(5) K_2SO_4	1	.036	.185	.084	.006
	2	.059	.190	.066	.020
	3	.043	.147	.031	.053
	4	.028	.154	.022	.061
	5	.017	.097	.010	.056
	6	012	.065	.006	.045
	7	.010	.049	.004	.038
	8	.006	.040	.004	.032

Potassium

Both with KCl and K_2SO_4, the amount of K lost in the first week was small. This gradually increased to a maximum in the fourth week and then decreased. The addition of nitrogenous salts to KCl increased the amount of potassium lost.

Magnesium Released from Successive KCl Applications

To investigate this point, periodic leaching and partial drying of

treatment 2 was continued past the initial 8-week period. Further KCl applications, each of 1 me.%K, were made at the end of the 8th and 14th weeks. The results are shown in Fig. 3. It was found that the amount of Mg released immediately following each KCl application formed a geometric progression being 0.155, 0.078, and 0.041 me.% after the first, second, and third KCl applications respectively. Amounts of calcium and sodium released showed no such progression.

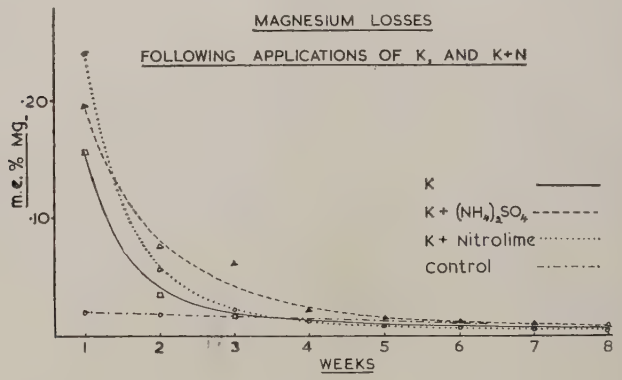

Fig. 1.

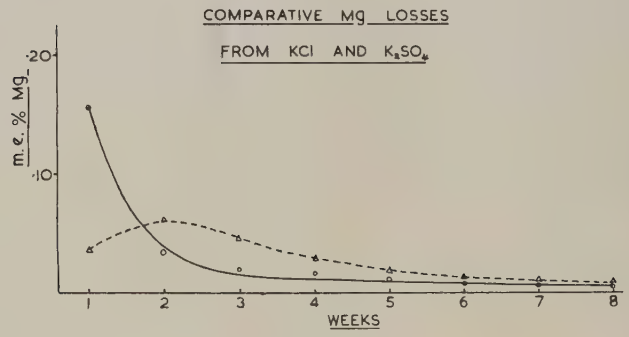

Fig. 2.

Decrease in Exchangeable Mg Levels

Three soils were examined for exchangeable Mg at the conclusion of the leaching period. These included the K_2SO_4 treated soil and two soils which had received repeated applications of KCl, and KCl + $(NH_4)_2SO_4$. Initial and final exchangeable magnesium levels, and amounts of Mg lost in drainage are shown in Table 2.

Field Evidence

In the laboratory investigation, the amount of water passing through the soil was 1 in. per leaching—a total of 16 in. over the

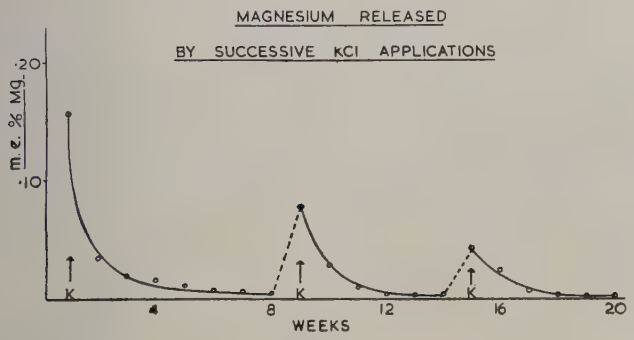

Fig. 3.

TABLE 2. DECREASES IN EXCHANGEABLE Mg LEVELS

Fertiliser Applied	Leaching Period	Exchangable Mg (me.%)		Mg Lost in Drainage (me.%)
		Initial	Final	
1 application K_2SO_4	8 weeks	.73	.55	.21
3 applications KCl	20 weeks	.73	.35	.45
3 applications KCl + $(NH_4)_2SO_4$	20 weeks	.73	.13	.69

eight-week period. This admittedly represents an extreme rate of leaching, 16 in. being approximately the amount of water lost in drainage in the Waikato in one year. Moreover, the laboratory study was carried out in the absence of any growing plants, and this raises the question of how far the results obtained can be related to field conditions.

A rates-of-potassium trial carried out at this Research Station on Horotiu sandy loam, provided opportunity for study of field data. This trial was laid down in 1955 and, with the exception of treatment 5 when the 1958 dressing was omitted, has had annual KCl applications of 0, 1, 2, 4, or 8 cwt per acre.

TABLE 3. EXCHANGEABLE Mg IN SOIL IN RATES-OF-K TRIAL

	Exch. Mg me.%	Significance Duncan's Test	
Control	1.24	a	A
1 cwt KCl	1.06	b	AB
2 cwt KCl	.94	b	BC
4 cwt KCl	.75	c	CD
8 cwt KCl	.69	c	D

Annual dressings of 5 cwt of superphosphate per acre have been applied to all plots, and there are 5 replications of each treatment. Plots have been mown periodically and clippings returned. 0–3 in. soil samples, taken in 1959, were examined for exchangeable magnesium. The mean of the values found is given in Table 3.

The 1 cwt KCl treatment has lowered exchangeable Mg by 0.18 me.%. Taking an acre of Horotiu sandy loam to a 3 in. depth as 470,000 pounds, an application of 90 pounds of dolomite (40% $MgCO_3$) per acre would be required to make good the magnesium lost over the 4 year period.

Discussion

Combined evidence from laboratory and field shows that magnesium losses follow applications of potassium chloride. No field evidence is available for trials with potassium sulphate, but the absence of an immediate Mg loss following the K_2SO_4 application in the laboratory suggests that plants would have more opportunity to take up the released magnesium and drainage losses should be less. Losses of Mg following application of the potassium as K_2CO_3 were also investigated in the laboratory and found to be negligible (the observation that, when K, Na or Mg are applied to the soil as the hydroxide or carbonate, the bases are absorbed without exchange, was made by Way in 1850). This is probably connected with the formation of the relatively insoluble calcium and magnesium carbonates and the weakly dissociated H_2CO_3, thus effectively reducing the number of cations available for further exchange in the soil.

In the case of the three soils examined for exchangeable Mg at the conclusion of the leaching period, the decrease in exchangeable magnesium was not greatly different from the amount lost in drainage. Thus, over the periods involved, there was no appreciable release of Mg from non-exchangeable sources.

This investigation points to the probable loss of some magnesium from the yellow-brown loams, following applications of KCl in the field. If the pattern of release in the field is similar to that found in the laboratory, decrease in exchangeable Mg levels should slow up as the level falls. There is no implication from the work carried out that magnesium deficiency is imminent. Nevertheless, it would be a wise precaution to carry out occasional analyses for exchangeable Mg on yellow-brown loams known to have a long history of potash top-dressing.

It is intended to use the laboratory technique described to investigate other soil types. From this study, we may at least be in a position to say where magnesium deficiency is most likely to develop first.

Acknowledgments

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A NOTE ON THE VARIATION OF INSULIN TOLERANCE IN CATTLE

By James R. Cook* and R. M. Gallagher, Ruakura Animal Research Station, Department of Agriculture, Hamilton

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Summary

The variation of insulin tolerance in normal cattle has been investigated using monozygotic twin Jersey heifers, and Aberdeen Angus bulls of four different sire groups.

Intravenous injections of protamine zinc insulin were made at the rate of 0.8 unit per kilogramme of body weight and the blood-sugar curve was followed over six one-hourly periods following injection.

The variation of insulin tolerance encountered between animals was mainly due to intangible environmental conditions. A small amount of the variation appeared to be due to genetic differences between animals.

It is concluded that the response of an animal to insulin stress is a character of low repeatability and is consequently of little value as a guide to a normal animal's potential rate of gain.

Introduction

The reaction of the blood sugar level to insulin injections in normal and dwarf beef cattle was studied by Foley *et al.* (1956). These workers concluded that the response of the two classes of cattle differed significantly, blood sugar levels returning to the original level much more rapidly in the normal animals. In order to facilitate this type of approach to a genetical problem, a study has been made of the nature of the variability of insulin tolerance in normal cattle using monozygous heifer twins; additional data were also obtained on four sire groups of young Aberdeen Angus bulls.

Materials and Methods

Ten pairs of identical-twin Jersey heifers, whose average weight was 512 ± 49 lb and whose average age was 493 ± 9 days, were submitted to the insulin tolerance test on 13 and 14 November 1958. These same animals were then submitted to the same test 21 days later, their average weight then being 534 ± 34 lb.

In the Gisborne area of New Zealand, some 250 miles from Ruakura, 14 Aberdeen Angus bulls on Farm A were submitted to the insulin tolerance test. Five days later 20 Aberdeen Angus bulls on Farm B were subjected to the same test. On Farm A there were eight bulls in one sire group and six in the other, while on Farm B there were ten bulls in each sire group. The average ages, weights, and gains are recorded in Tables 1 and 2.

* Fulbright Fellow: Present address: Department of Animal Husbandry, University of California, Davis, California, U.S.

TABLE 1. Average Ages and Weights of Angus Bulls

Farm	Sire	n	Age at Weaning	Weight at Weaning	Age at Test	Weight at Test
A	M	6	238 ± 7	520 ± 7	499 ± 7	956 ± 32
	T	8	238 ± 9	547 ± 29	499 ± 9	966 ± 35
Total		14	238 ± 8	536 ± 31	499 ± 8	926 ± 34
B	L	10	255 ± 21	463 ± 45	519 ± 21	746 ± 41
	J	10	249 ± 17	434 ± 55	514 ± 17	646 ± 76
Total		20	252 ± 19	448 ± 58	517 ± 19	696 ± 79

TABLE 2. Average Gains of Angus Bulls

Farm	Sire	n	Weaning Date	Gain from Weaning to 11.9.58	Gain from 11.9.58 to 22.1.59	Gain from 22.1.59 to 22.5.59	Total Gain
A	M	6	7.5.58	126 ± 28	310 ± 24	143 ± 18	579 ± 47
	T	8	7.5.58	124 ± 13	195 ± 44	119 ± 33	538 ± 59
Total		14		125 ± 22	301 ± 37	129 ± 30	555 ± 57
				Weaning to 12.9.58	from 12.9.58 to 27.1.59	from 27.1.59 to 21.5.59	
B	L	10	8.5.58	17 ± 34	266 ± 23	158 ± 30	441 ± 55
	J	10	8.5.58	—9 ± 25	221 ± 30	127 ± 35	339 ± 58
Total		20		4 ± 30	244 ± 60	143 ± 37	391 ± 76

All cattle were injected with 0.8 units of protamine zinc insulin per kilogramme of body weight. The blood was collected from the right external jugular vein into 25-ml blood bottles that had been previously prepared with potassium oxalate as an anticoagulant, and sodium fluoride as a preservative. Approximately 20 ml of blood was run into each bottle and gently shaken for one to two minutes to dissolve and mix the anticoagulant and preservative with the blood. Following the first blood sample the insulin was immediately injected. Hourly samples were then taken for a period of six hours.

The blood was deproteinised within one hour of collection by the method of Folin and Wu (1919), and the protein-free filtrate was stored overnight at 34–36°F. The following day the glucose determinations of

the samples were obtained by the modified Folin and Wu method (Folin and Wu 1920; Folin 1926, 1929). The blood collected in the Gisborne area was handled in the same way with the exception that it was shipped on ice to the Ruakura laboratory, delaying the deproteinisation by approximately 48 hours from the time of collection.

Results

The difference between the period means obtained when monozygotic-twin Jersey heifers were submitted to the insulin tolerance test on two occasions (Table 3) was found to be significant at the initial, first, second, fifth, and sixth hourly samples. Between-pair differences were significant at the second, third, fifth, and sixth hourly samples (Table 4).

TABLE 3. Means of Glucose Levels of Monozygotic Twins Subjected to Insulin Tolerance at Two Different Periods

Hour	First Period n = 20	Second Period n = 20
0	84.09	71.99
1	56.69	34.96
2	53.32	47.11
3	51.79	57.15
4	58.59	65.78
5	60.22	72.40
6	57.89	69.85

On Farm A the two sire groups of Aberdeen Angus bulls had been fed from weaning a concentrate supplement plus good pasture *ad lib.* These two groups had an average difference of six pounds of body weight at the time of the test and an average difference of gain of 41 lb from weaning on 22 May 1959 (Table 2). The differences between the two groups in blood-sugar level after insulin injection were slight (Table 5).

On Farm B there was an average difference between the two sire groups of 100 lb in body weight and an average difference in gain of 102 lb from weaning to 21 May 1959 (Table 3). The management practices on this farm were rather typical of beef cattle management in New Zealand where the cattle are raised on grass over the winter period, being fed only enough hay to maintain their body weight, and never receiving any concentrate supplement through their life. The differences in insulin tolerance in these two groups were of much greater magnitude than on Farm A, but not significant statistically.

Discussion

The analysis in Table 3 shows quite clearly that the response of an animal to insulin injection at any one time is unlikely to be a repeatable characteristic of that animal. In other words, temporary environmental effects play a greater part in determining the variation between

TABLE 4. Mean Squares of Glucose Levels of Monozygotic-twin Heifers Subjected to Insulin Stress

Hour	*df*	0	1	2	3	4	5	6
Period	1	1462.28**	4722.37**	1255.41**	287.89	517.53	1489.13**	1431.85**
Pairs	9	137.82	162.63	266.47*	1312.79**	279.43	547.39**	424.71*
Within	29	63.32	134.56	89.27	113.47	150.67	147.18	171.16

* 5%. ** 1%.

TABLE 5. Means and Standard Error of the Mean of Glucose Levels of Aberdeen Angus Bulls Subjected to Insulin Stress

Farm	Sire	0	1	2	3	4	5	6
A	M	58.95 ± 1.88	39.60 ± 5.05	41.52 ± 3.71	41.43 ± 3.06	31.75 ± 2.89	39.95 ± 2.18	41.35 ± 2.79
	T	59.71 ± 3.12	35.42 ± 2.96	36.79 ± 2.61	37.44 ± 2.54	38.94 ± 2.79	39.49 ± 2.29	41.69 ± 2.27
	Mean	59.34 ± 1.90	37.21 ± 2.69	38.81 ± 2.18	39.15 ± 1.96	40.14 ± 1.98	39.68 ± 1.55	41.54 ± 1.70
B	L	56.61 ± 1.76	33.61 ± 1.81	37.14 ± 2.98	44.27 ± 3.30	48.36 ± 3.47	54.24 ± 4.20	53.40 ± 4.61
	J	57.92 ± 1.31	28.10 ± 2.77	34.13 ± 3.52	40.21 ± 3.07	45.87 ± 3.47	46.39 ± 2.85	45.03 ± 3.48
	Mean	57.26 ± 1.08	30.86 ± 2.18	35.64 ± 2.27	42.24 ± 2.24	47.12 ± 2.46	50.32 ± 2.62	49.22 ± 2.97

animals to insulin than do genetic differences. The reason for the difference in nature of the response of the cattle on Farm A compared with those on Farm B can only be guessed at. What is clear, however, is that in order to distinguish significant sire-group differences in the response to insulin very large progeny groups would be required.

Acknowledgments

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ATTEMPTS TO PRODUCE HYPOMAGNESAEMIA IN DRY NON-PREGNANT SHEEP

By K. R. Christian and V. J. Williams, Ruakura Animal Research Station, Hamilton

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Summary

Dosing urea or ammonium acetate (up to 11 g ammonium nitrogen equivalent) failed to lower serum magnesium or calcium in wethers fed a ration of dried grass. This level of dosing raised blood non-protein nitrogen by 4–14 mg%, and was sufficient to cause inappetance within several days.

Serum magnesium of three groups of sheep which had been fed in sheltered pens on fresh grass, dried grass, and dried grass plus ammonium acetate respectively fell within one day after the sheep were turned out to spring pasture, but were normal on the fourth day of grazing.

When sheep that had been free grazing were starved in sheltered pens, serum magnesium rose within one day but had returned to the original level after four days. Serum calcium showed successive falls during this time.

Linear falls in serum magnesium and calcium were produced when sheep, both in lean and in fat condition, were starved for several days in a pen exposed to the weather. When the sheep were let out to pasture again, serum calcium returned to normal in three days but serum magnesium fell still further and was still low after four days.

Acute hypomagnesaemia without accompanying hypocalcaemia was not produced by under-nutrition or starvation; it is concluded that the observed changes in magnesium levels were associated with the change from pens to pastures.

Introduction

Several types of hypomagnesaemia have been distinguished, but this paper will deal only with the acute form encountered on rapidly growing pastures. The condition has not been definitely correlated with a deficiency of dietary magnesium, and it may appear under a variety of weather and soil conditions and botanical composition of the pasture (Allcroft 1955). Low serum magnesium levels do not necessarily bring about tetany, which seems to result from some other trigger mechanism, but it is well recognised that hypomagnesaemia is conducive to tetany and should receive primary attention in elucidation of the disorder.

Two possible causes have been investigated. Firstly, Head and Rook (1955) have reported a fall in serum magnesium within four days following the introduction of ammonium salts into the rumen of cows and have suggested that the high levels of ruminal ammonia found in cattle on flush pasture growth might in some way be connected with hypomagnesaemia. This effect, if uncomplicated, should be readily verifiable.

Secondly, Swan and Jamieson (1956) have shown that a number of factors liable to result in digestive upsets or sudden falls in feed intake

TABLE 1. TRIAL 1: DOSING AMMONIUM ACETATE AND UREA

Period	Days on Treatment	Basal Ration (g/day)	Dosing Treatment (gNH_3-N/day) Gr.A	Gr.B	Sampling Day	Blood Findings (mg/100 ml) Serum Magnesium Gr.A	Gr.B	Serum Calcium Gr.A	Gr.B	Blood N.P.N. Gr.A	Gr.B
1	10	Hay—500	—	—	4	2.2	2.1	9.5	10.0	—	—
					6	—	—	9.4	10.1	—	—
					10	2.0	1.8	10.2	10.1	—	—
2	5	Dried Grass—400	—	—	—	—	—	—	—	—	—
3	12	Dried Grass—400	—	Urea 7.5	2	2.1	1.7	9.9	10.3	—	—
					4	2.1	1.9	9.8	10.1	—	—
					9	2.4	2.1	10.1	10.0	33	41
					11	2.4	2.1	9.9	10.2	37	41
4	3	Dried Grass—400	—	—	—	—	—	—	—	—	—
5	4	Dried Grass—400	Urea 11.2	—	2	2.3	2.1	—	—	42	33
					4	2.4	2.2	—	—	44	38
6	4	Dried Grass—400	—	—	—	—	—	—	—	—	—
7	4	Dried Grass—400	Acetate 11.6	—	2	2.6	2.3	10.0	10.3	36	50
					4	2.6	2.3	10.1	10.5	31	45
8	12	Dried Grass—400	—	—	7	2.4	2.3	9.8	10.6	33	32
9	10	Dried Grass—200	—	Acetate 5.0	2	2.4	2.1	9.7	10.1	38	43
					4	2.5	2.1	10.0	9.9	35	42
					9	2.4	2.1	9.4	9.6	40	48
					11	2.4	2.1	9.4	9.8	37	48
10	4	Free Grazing	—	—	2	1.7	1.5	9.0	9.4	47	49
					4	2.6	2.2	9.8	9.8	46	51
11	3	Free Grazing	Acetate 10.1	—	4	2.8	2.7	9.1	8.8	54	55

can produce clinical cases of staggers and that cows that were high producers, in good condition, or in the early stages of lactation were most susceptible. Cases of tetany often occur in Europe when stock that have been housed for the winter are turned out to spring pasture, incurring extreme changes both in diet and in living conditions. A closer study of these findings was thought likely to be profitable in distinguishing between the factors likely to be involved.

Though termed "lactation tetany", clinical cases are not confined to cattle undergoing the stress of pregnancy or lactation. Cases of affected ewes have been reported in Scotland (Stewart 1954) but none to our knowledge in New Zealand. It was decided to use mature, dry, non-pregnant sheep for the present trials because of their low physiological requirements and their convenience for experimental purposes.

Experimental and Results

Methods

Blood samples were collected from the jugular vein before the morning feed in the case of pen-fed animals and between 0800 and 1100 hours for grazing animals. Analytical methods for serum magnesium and calcium and blood non-protein nitrogen and eosinophils were as used previously (White *et al.* 1956). Serum potassium and sodium were determined by flame photometry. Ruminal ammonia was determined by the method of Conway (1947).

Because of the necessarily short-term nature of each of these trials, it has not been practicable to carry out rigorous statistical analyses of the results in most cases.

Trial 1. Ammonia Dosing

Eight mature wethers were divided into two equal groups, A and B. These animals had been individually penned and fed a maintenance ration of fresh grass for the previous 60 days.

The treatments are listed below under consecutive periods; other experimental details and results are summarised in Table 1.

Period 1

Both groups were fed chopped hay for a preliminary period of 10 days, but the ration proved to be of low palatability, and live weights fell from an average of 84 lb to 79 lb.

Period 2

The ration was changed to 200 g good quality dried grass (dry matter 85%) twice daily for a preliminary period of five days.

Period 3

Group B were dosed twice daily after feeding with 8 g urea in water as a source of ammonia. Ruminal ammonia levels 2 hours after the morning dosing were 30 mg% for Group A and 75 mg% for Group B. There were few feed residues during the twelve days of treatment.

Period 4

Both groups were fed dried grass only for three days.

Period 5

Group A were dosed 6 g urea in water four times daily. After three days, three of the dosed sheep lost appetite almost completely and dosing had to be stopped.

Period 6

Both groups fed dried grass only for four days. Appetites quickly returned to normal.

Period 7

Group B were dosed 16 g ammonium acetate in water four times daily; after three days, two dosed sheep completely refused feed, and dosing was stopped.

Period 8

Both groups on dried grass only for twelve days. Appetites quickly returned to normal.

Period 9

In order to determine the effect of high ruminal ammonia at low intake levels, the ration of both groups was reduced to 100 g dried grass twice daily. In addition, Group B were dosed 14 g ammonium acetate in water twice daily after feeding. All feeds were completely eaten during the ten days of treatment. Live weights, which had remained steady since period 1, fell by an average of 6 lb.

Period 10

Both groups were let out on to rapidly growing spring sheep pasture at 1000 hours. All sheep began grazing immediately. Weather conditions were mild. After four days at pasture, ruminal ammonia levels averaged 41 mg% at 1000 hours.

Period 11

After the sheep had been at pasture four days, Group A were dosed 28 g ammonium acetate in water twice daily. Ruminal ammonia levels one hour after the morning dosing average 79mg% for Group A as compared with 42mg% for Group B.

It will be seen from Table 1 that none of the dosing treatments affected serum calcium or magnesium levels to any significant degree. Though serum magnesiums were low at the beginning of Period 3, possibly as the result of under-nutrition in Period 1, a gradual recovery took place in both groups, whether dosed urea or not. Dosing ammonium salts when the ration was below maintenance requirements (Period 9) was also ineffective. While the possibility still exists that pen-fed animals on a high plane of nutrition dosed a proportional amount of ammonia might show some response, high ruminal ammonia levels in sheep on ample pasture did not appear to produce any short-term effects. On the other hand, the fall in serum magnesium 24 hours after letting out to pasture ranged between 0.6% and 0.9%, though the recovery was complete in all sheep after another three days, and in fact rose subsequently to levels higher than those found at any time in the pens.

Dosing with ammonium acetate and urea in all cases raised blood non-protein nitrogen levels by an average of 4–14 mg%—that is, to values approaching those at pasture.

Trial 2. Change from Sheltered Pens to Pasture

Twelve mature wethers and dry ewes were individually penned indoors and fed fresh grass for 40 days. They were then shorn and divided into three groups of four sheep each. The first group was fed

TABLE 2. TRIAL 2: PEN-PASTURE
(average pen treatments)

Treatment	Sampling Day	Dried Grass (300g/day)						Dried Grass (1000g/day)						Fresh Grass (2000g/day)					
		Serum Mg		Serum Ca		Eosinos		Serum Mg		Serum Ca		Eosinos		Serum Mg		Serum Ca		Eosinos	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Pens	1	2.4	0.1	9.6	0.5	—	—	2.0	0.3	9.8	0.2	-	—	2.2	0.2	9.8	0.2	-	—
	7	2.2	0.3	10.6	0.1	390	240	1.9	0.5	9.6	0.4	60	30	2.0	0.4	10.2	0.3	460	170
Pasture	8	1.8	0.4	10.0	0.3	230	220	1.3	0.3	9.6	0.3	50	40	1.6	0.5	9.7	0.2	200	80
	11	2.2	0.2	10.1	0.5	250	180	2.1	0.3	10.2	0.4	70	30	2.1	0.5	10.1	0.4	240	180

300 g dried grass a day, the second group 1000 g dried grass a day, and the third group 2000 g fresh grass a day. This treatment lasted seven days. Two sheep from each group were then let out on to short, yellowish, sheep pasture regrowth, while the other two from each group were released on to long, coarse, mature dairy pasture.

Results grouped according to pen-feeding treatments are shown in Table 2 and according to pasture treatments in Table 3. The fall in serum magnesium after 24 hours at pasture ranged from 0.1 to 1.4 mg% and four values of 1.2 mg% were recorded. Levels rose in all but two sheep within the next three days, when all values were above 1.5 mg%, the accepted lower limit of normality. No difference appeared to have been brought about by the different feeding treatments either in pens or at pasture. No significant changes in serum calcium were found. Eosinophil levels showed that when in the pens the sheep were in a condition of stress which was not changed by letting out to pasture.

TABLE 3. Trial 2: Pen-pasture
(average pasture treatments)

Treatment	Sampling Day	Sheep Pasture						Dairy Pasture					
		Serum Mg		Serum Ca		Eosinos		Serum Mg		Serum Ca		Eosinos	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Pens	1	2.2	0.4	9.7	0.2	—	—	2.2	0.3	9.7	0.4	—	—
	7	2.2	0.4	10.3	0.5	440	550	1.8	0.4	10.0	0.5	370	470
Pasture	8	1.7	0.5	9.9	0.4	290	380	1.5	0.7	9.6	0.4	200	160
	11	2.2	0.3	10.4	0.2	200	250	2.0	0.3	9.9	0.5	390	530

Trial 3. Starvation in Sheltered Pens

Three groups of four dry non-pregnant ewes in good condition were grazed on short sheep pasture, on mature dairy pasture and on very short green pasture respectively. After eleven days' grazing, all sheep were shifted to individual pens which were under cover and protected from the north side. Water only was provided for four days.

Results are presented in Table 4. The rise in serum magnesium after 24 hours in pens ranged from 0.1 to 1.1 mg%, while levels after a further three days starvation were in general comparable with the levels at pasture, with the exception of those for the group on very short pasture which were somewhat lower. Calcium levels fell in all but two sheep by 2.1 to 3.6 mg% during the four days of starvation, most of this fall taking place after 24 hours; this effect was similar to that found when penned sheep that had been fed on dried grass were starved (White *et al.* 1956).

Trial 4. Thin Sheep Starved in Exposed Pens

Ten mature sheep (wethers and dry ewes) that had been grazed on rank spring pasture were locked in a pen 10 ft long and 5 ft wide.

TABLE 4. Trial 3: Sheep Fasted in Sheltered Pens
(average 4 sheep)

Treatment	Sampling Day	Sheep Pasture						Dairy Pasture						Very Short Pasture					
		Serum Ca		Serum Mg		Eosinos		Serum Ca		Serum Mg		Eosinos		Serum Ca		Serum Mg		Eosinos	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Pasture	1	9.8	0.3	2.2	0.3	—	—	9.6	0.2	2.2	0.1	—	—	9.1	1.3	2.7	0.3	—	—
	7	9.8	0.5	2.4	0.3	880	560	9.6	0.6	2.7	0.3	840	270	8.7	0.7	2.8	0.1	1060	470
Fasted 4	8	8.2	0.4	3.4	0.3	670	180	7.6	0.3	3.0	0.3	600	240	8.0	0.8	3.0	0.2	1100	200
days in sheltered pens	11	7.7	1.8	2.4	0.4	740	260	7.7	0.4	2.5	0.2	520	350	5.9	0.8	2.1	0.1	900	320

TABLE 5. Trial 4: Thin Sheep Fasted in Exposed Pens
(average 10 sheep)

Treatment	Sampling Day	Live-weight (lb)	Serum Mg mg%		Serum Ca mg%		Serum K mg%		Serum Na mg%		Eosinos per cu.mm.	
Free grazing			Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
	1		2.2	0.5	10.2	0.4	21.3	1.0	358	21	—	—
	6	90	2.6	0.6	10.6	0.3	19.8	1.1	365	11	520	420
	7		2.7	0.5	9.6	0.5	23.7	1.2	348	13	460	310
Fasted in	8		2.3	0.5	8.6	0.5	20.4	0.9	333	7	—	—
unsheltered	9		2.1	0.7	8.2	1.2	18.6	1.3	337	9	—	—
pen	10	70	1.9	0.6	7.8	1.0	20.5	1.4	338	12	—	—
	11		1.7	0.4	9.1	0.7	23.4	2.1	330	10		
Free grazing	12		1.5	0.6	10.0	1.0	21.9	0.9	347	23	—	—
	13		1.6	0.6	9.9	0.6	22.5	0.9	373	15	—	—
	14	84	1.7	0.6	9.5	0.7	22.2	1.2	343	17	—	—

They were provided with water, but had no access to feed for four days, after which they were returned to pasture. The weather during this time was cold and windy, with occasional showers.

Results are presented in Table 5. A statistically significant difference between the three phases of the experiment was found in both serum magnesium and calcium. The fall during starvation was linear for both constituents. By contrast with Trial 3, no appreciable rise in serum magnesium was found after one day's starvation. On release to pasture, the recovery in serum calcium was very rapid in all sheep, whereas serum magnesium showed a further fall within two days of returning to pasture in all but one sheep. Levels at this time were 0.6–2.3 mg% below those prior to locking up. Several values of 1.2 mg% or less were recorded, and recovery was by no means complete after four days at pasture. No explanation can be offered for the transient rises in serum potassium on the first day of locking up and on letting out. Bartlett *et al.* (1957) found no changes in serum sodium, potassium, or calcium when cattle were turned out on to potentially tetanic pastures. The rather low eosinophil levels observed prior to locking up suggest that the animals were in a condition of moderate stress when the trial started.

Trial 5. Fat Sheep Starved in Exposed Pens

Ten sheep (mature wethers and dry ewes) that had been grazed on long spring pasture were locked for five days in the same pen as was used in Trial 4. Owing to being inadvertently without water on the last two days of starvation the sheep were moderately thirsty on being returned to pasture.

Results are presented in Table 6. The changes in serum calcium and magnesium were very similar to those found in Trial 4. Serum magnesium reached the lowest point one day after the sheep were

TABLE 6. TRIAL 5: FAT SHEEP FASTED IN EXPOSED PENS
(average 10 sheep)

Treatment	Sampling Day	Live Weight (lb)	Serum Mg mg%		Serum Ca mg%	
Free grazing			Mean	sd	Mean	sd
	1		2.4	0.5	10.7	0.4
	2	151	2.9	0.5	10.6	0.5
Fasted in pen	3		2.4	0.4	9.6	1.2
	4		2.0	0.3	8.6	1.3
	7	127	1.8	0.2	9.0	0.9
Free grazing	8		1.6	0.3	8.8	1.6
	9		1.7	0.4	8.8	1.4
	10		2.1	0.3	10.7	1.0
	11		2.0	0.4	11.3	0.8

released to pasture, being then 0.5–1.3 mg% lower than before locking up. The fall in serum calcium during starvation was less than in Trial 4, and the return to normal required three days at pasture.

Discussion

The lowering of both serum calcium and magnesium levels concurrently occurs with moderate reduction of intake of dried grass (Williams and Christian 1959); when sheep in pens are starved (White *et al.* 1956); or when sheep that have been free grazing are starved in exposed conditions (Trials 4 and 5). However, the changes in serum magnesium that are of primary interest in the present study are those that are unaccompanied by similar changes in serum calcium. This took place only when sheep were transferred from pens to pasture.

At least four factors would seem to be involved; change in diet, increased feed intake, exposure to weather conditions and greater opportunity for exercise. Since the sheep in pens fed fresh grass in Trial 2 showed similar falls in serum magnesium to those fed dried grass, change in the nature of the diet could have made little contribution to this effect. No evidence has been produced, however, to show whether increased feed intake lowers serum magnesium, particularly in animals in very poor order as were used in these trials, though this seems unlikely.

As far as is known, the rise in serum magnesium on the first day of starvation in sheltered pens in Trial 3 is an effect that has not been reported before. It is at variance with that produced by starvation in the open (Trials 4 and 5), which suggests that magnesium requirements are greater in animals unprotected from wind and cold. In this connection it has been stated that a sudden spell of wet, cold weather may precipitate clinical cases and that strong winds may lower serum magnesium (Swan and Jamieson 1956).

Trials 4 and 5 were conducted to test the possible interaction between exposure and starvation on serum magnesium. It was also hoped that sheep whose magnesium levels had already been lowered by starvation might suffer a further fall when allowed to graze pasture again and this attempt was partly successful. The behaviour of serum magnesium was in marked contrast with that of calcium only when the animals were released to pasture, suggesting that starvation, even under inclement conditions, is inadequate to cause uncomplicated hypomagnesaemia. There is no reason to suppose that any impairment in feed intake on resumption of grazing is responsible for the further fall in magnesium. Since the sheep had only a restricted amount of movement when penned, a study of magnesium requirements for exercise would be of value.

While total starvation for several days is most unlikely in field cases, it is conceivable that animals with greater physiological demands, as in pregnancy or lactation, may react more strongly to a less severe nutritional check. White (1954) has shown that lower serum magnesium levels prevail in dairy cows under uncontrolled grazing conditions, and these animals may be more subject to tetany. However, no

particular differences were noted between the reactions of the thin sheep in Trial 4 and those of Trial 5 with abundant body reserves.

Some degree of hypomagnesaemia without accompanying hypocalcaemia may therefore be produced in dry non-pregnant sheep merely by turning them from pens to pasture. Change of diet alone, and higher production of ruminal ammonia in particular, does not seem to account entirely for this effect, which would appear to be due to changes in environmental conditions. A variety of trials involving permutations of such changes in conditions immediately suggest themselves and appear to merit investigations.

Acknowledgments

We wish to express our appreciation to Mr J. E. Allan of the Rukuhia Soil Fertility Station for the sodium and potassium determinations, to Misses N. G. Young and V. Shanks for calcium and magnesium determinations, and to Mr P. P. McKitterick for general technical assistance.

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THE INFLUENCE OF NITROGEN ON SEEDLING AND EARLY GROWTH OF PERENNIAL RYEGRASS AND COCKSFOOT

By T. A. O'Brien, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 11 January 1960*)

Summary

The growth of seedlings of perennial ryegrass (*Lolium perenne* L.) and cocksfoot (*Dactylis glomerata* L.) was studied at different soil nitrogen levels.

At all nitrogen levels, and at all stages of growth studied, perennial ryegrass seedlings produced more dry matter than cocksfoot.

After the initial two-weeks period, the dry-matter production ratio of perennial ryegrass to cocksfoot was greater at the lower levels of nitrogen than at the higher.

At high nitrogen levels the relative growth rates of the two species were similar, but at lower nitrogen levels the relative growth rate of perennial ryegrass was significantly greater than that of cocksfoot in the second two weeks of growth, and significantly less than that of cocksfoot in the fourth two weeks of growth.

The results are discussed in relation to early competition as affected by soil nitrogen, light, and temperature, between these two species in pasture mixtures.

Introduction

In New Zealand, attempts to establish mixtures of perennial ryegrass (*Lolium perenne* L.) and cocksfoot (*Dactylis glomerata* L.) in soils of high fertility, are often unsuccessful because the ryegrass suppresses the cocksfoot. Where available nitrogen is at intermediate or lower levels perennial ryegrass seedlings appear to compete less strongly with those of cocksfoot. This difference appears to be greater with autumn sowings than with spring sowings.

Pasture species have been classified by various workers according to their seedling aggressiveness. Stapledon and Davies (1927) and Blaser *et al.* (1956), on the criterion of relative weights of seedlings at some time after sowing and grown under conditions of high fertility, rated perennial ryegrass as being more aggressive than cocksfoot. Factors discussed by these workers as contributing to this seedling aggressiveness included seed size, rate of germination and rate of seedling growth.

Cashmore (1934) investigated some factors of the aggressiveness of perennial ryegrass over *Phalaris tuberosa* L. in high fertility soil in the first growing season. Using both single plants and mixtures in plots, he concluded that the depressing effect of ryegrass on phalaris in the seedling year was due to the greater top growth of the former combined with its denser rooting system in the upper foot of soil.

Competition for light and nutrients under conditions of liberal water supply have been demonstrated by several workers to be the contributing factors to a species aggressiveness during seedling growth. Chippindale (1932) attributed the aggressiveness of Italian ryegrass (*Lolium multiflorum* L.) over meadow fescue (*Festuca pratensis* L.) to this, and Donald (1958) concluded that the same factors (the nutrient in this case being nitrogen) enabled perennial ryegrass to be the aggressor species over phalaris. Furthermore, Donald showed that the combined effect of competition for both factors exceeded the sum of the separate effects.

This present study was undertaken to determine the influence of soil nitrogen level on the comparative growth of perennial ryegrass and cocksfoot at varying stages of their seedling and early growth.

Experimental

The experiment was carried out at the Palmerston North station of Grasslands Division, Department of Scientific and Industrial Research.

Fig. 1.—One block of the layout, showing 10 randomised plots and two sub-plots per plot for the two species. The temperature recording unit is also shown.

The experimental site was divided into six blocks each measuring 21 ft x 3 ft (see Fig. 1).

Each block was subdivided into ten plots to provide a total of 60 plots, each measuring 2 ft x 3 ft. The soil used for all plots was Taupo pumice alluvium (Fleming 1953). This soil type (total nitrogen 0.02%) was chosen following a preliminary glasshouse experiment designed to determine the degree of plant response to nitrogen treatment in different soil types.

Each plot was divided into two sub-plots one of which contained two rows of perennial ryegrass and the other two rows of cocksfoot, allowing six inches between all rows within the plot. Sixteen uniformly germinated seeds of both species were sown per row at two-inch spacings and at $\frac{1}{2}$ in. depth, on 21 November 1958.

To obtain common emergence time and physiological age at transplanting, it was necessary, from the results obtained in a preliminary investigation on the differences in rate of germination between these two species, to pre-germinate the cocksfoot seed two days before the ryegrass. This was done on sand in petri dishes (in half-strength Hoagland's solution) and seed was then selected for uniformity, at the stage of emergence of the coleorhiza. The term "germinated seeds" referred to hereafter denotes this stage, when actual planting took place.

Dry weights of germinated seeds were determined by drying and weighing ten groups (each of one hundred) of uniformly germinated seeds for each species.

Nitrogen treatments applied were as follows:—

1, 2, 3	CONTROL
4, 5, 6	6 lb N/ac.
7, 8	20 lb N/ac.
9	60 lb N/ac.
10	120 lb N/ac.

Nitrogen was applied in the form of ammonium nitrate (35% N).

These were laid down as a 10 × 2 split-plot design, the additional replicates within blocks being used to provide greater accuracy in measurement at the lower levels of N. The numbers of plants per harvest for each treatment were therefore: 144 for Control and 6 lb N/ac, 96 for 20 lb N/ac, and 48 for 60 and 120 lb N/ac.

Superphosphate at 5 cwt/ac, and magnesium sulphate and muriate of potash at 1 cwt/ac, were applied to all plots. The superphosphate was applied five days prior to sowing and the quantity to be given per plot was bulked with pumice and spread evenly, at approximately $\frac{1}{2}$ in. depth along the drill rows. Both the muriate of potash and magnesium sulphate were applied in solution two days prior to sowing.

Nitrogen was applied in split applications in the following way. One tenth of the whole of each treatment was added one day prior to sowing. One day subsequent to seedling emergence for both species, one twentieth of the whole of each treatment was applied in solution and additions at this rate were repeated with such frequency and at regular time intervals, that by the end of the third wek all nitrogen treatments had received half of their proposed application. The balance of the applications were made twice weekly, again at the rate of one twentieth of the whole treatment, for the remainder of the experimental period, the last application being given four days prior to the final harvest.

All plots were watered at frequent intervals throughout the experimental period.

Four harvests were made at fortnightly intervals, the first being made fourteen days after mean seedling emergence. There was a delay of approximately one day in cocksfoot emergence, but this has been ignored. At each harvest date, eight plants were harvested from each sub-plot, four being taken from each row, and bulked. These plants were selected from a restricted random plan in such a way that after the second harvest the distance between the plants was doubled.

At each harvest, the plants from each sub-plot were washed, roots trimmed off, and the remainder bulked in air-tight containers and put in cool storage. After determination of tiller numbers, leaf numbers, and leaf areas, the shoots were oven-dried and weighed.

The growth increments between harvests were determined, the seed weights being used to determine the increments at the first harvest. The results were then expressed in terms of relative growth rates (Blackman 1919).

The method of determining leaf area was as follows. Twenty plants of each species from each treatment were selected at random at each harvest. All leaves from each plant were severed at the position of the ligule, and from these a number of both mature and immature leaves were selected. At the middle of each selected leaf, a 2 cm section was cut and its width measured, the rest of the leaf being bulked with the remaining leaves for each plant. Both the bulk of the leaf material, the strips and the stems were oven-dried and separately weighed. The total leaf area for each plant was then calculated.

Results

Weather Conditions

Mid November to mid January, a period with approximately a 16-hour day length, was on the whole fine and sunny. The means of the daily maximum and minimum temperatures (°F) for the period were:—

	6 in. above	Ground Surface	1 in. below	3 in. below
Maximum	78.4	82.5	74.9	71.8
Minimum	52.9	52.7	54.1	59.5

TABLE 1. Dry Weights (mg) of the Shoots of Single Perennial Ryegrass and Cocksfoot Plants as Influenced by Nitrogen Levels at Four Harvest Dates. Ryegrass/Cocksfoot Ratios are Shown in Italics

Treatment (Equivalents of Applied Nitrogen in lb N per acre)	Harvest 1: 9/12/58		Harvest 2: 23/12/58		Harvest 3: 6/1/59		Harvest 4: 20/1/59	
	Perennial Ryegrass	Cocksfoot	Perennial Ryegrass	Cocksfoot	Perennial Ryegrass	Cocksfoot	Perennial Ryegrass	Cocksfoot
Nil	2.48 ± 0.06 *2.1*	1.20 ± 0.03	14.4 ± 0.5 *3.6*	3.98 ± 0.21	46.1 ± 2.1 *5.7*	8.0 ± 0.4	115 ± 6 *4.3*	26.8 ± 1.3
6	3.26 0.08 *2.0*	1.61 0.04	23.8 0.8 *3.6*	6.61 0.21	72.6 3.4 *3.1*	23.3 1.1	177 9 *2.4*	73.1 3.5
20	5.09 0.17 *2.3*	2.24 0.08	49.8 2.0 *2.5*	20.2 0.8	159 9 *2.0*	79 5	352 21 *1.4*	258 16
60	9.1 0.4 *2.3*	3.90 0.18	130 7 *2.4*	53.6 3.0	637 49 *2.2*	290 23	2150 134 *1.7*	1274 109
120	12.5 0.6 *2.8*	4.40 0.20	209 12 *3.0*	68.7 3.8	1222 96 *1.9*	628 49	4365 372 *1.8*	2489 212
Coefficient of variation (per 8 plants)	10.8%		4.5%		4.6%		3.9%	

These recordings were obtained from a recording unit sited in the plots (see Fig. 1). The maximum temperature recorded, which was at the soil surface, was 102.5°F on 11 January. During the same day the maxima recorded for six inches above the soil surface, one inch below and three inches below were 90.5°F, 86°F and 82.5°F respectively.

The minimum soil temperature recorded was 33°F on 21 November. The minima at the soil surface, one inch and three inches below the soil surface during this same day were 34°F, 38°F and 48°F respectively.

Seed Weights

The total dry weights (g) of one thousand germinated seeds, together with standard errors, were:—

Perennial ryegrass	2.09 ± 0.03
Cocksfoot	0.804 ± 0.005

Herbage Yields (Dry Matter)

For each harvest, coefficients of variation were stable over the five treatments. The data analysed are therefore logarithms of mean weights (mg) per plant. The interpretations were made from the analysis of variance and transformed means, but in the table of means presented in Table 1 the decoded means and standard errors are given.

The following are the significant features of Table 1:

(a) Perennial ryegrass outyielded cocksfoot in all treatments at all harvest dates.

(b) There were production increases from added nitrogen for both species at all harvests.

(c) There were significant species x nitrogen level interactions at all harvests, i.e., the yield ratio of ryegrass to cocksfoot changed significantly with nitrogen level at all harvests. Table 1 also shows that the absolute difference in favour of perennial ryegrass increased progressively with nitrogen level with no sign that the difference between the species had reached its maximum at 120 lb N/ac.

Tiller Number

Tiller numbers for the four harvest dates are presented graphically (log. scale) in Fig. 2. Average S.E.s are also shown for each harvest.

The following comparisons are of interest:

(a) At all harvest dates, and at all nitrogen levels, there were species differences in tiller number in favour of perennial ryegrass. The only exception was at the control level in the first harvest, in which the difference did not reach significance.

(b) At all harvests, there were significant species x nitrogen interactions, the absolute increase in tiller number with increased nitrogen being significantly greater in perennial ryegrass.

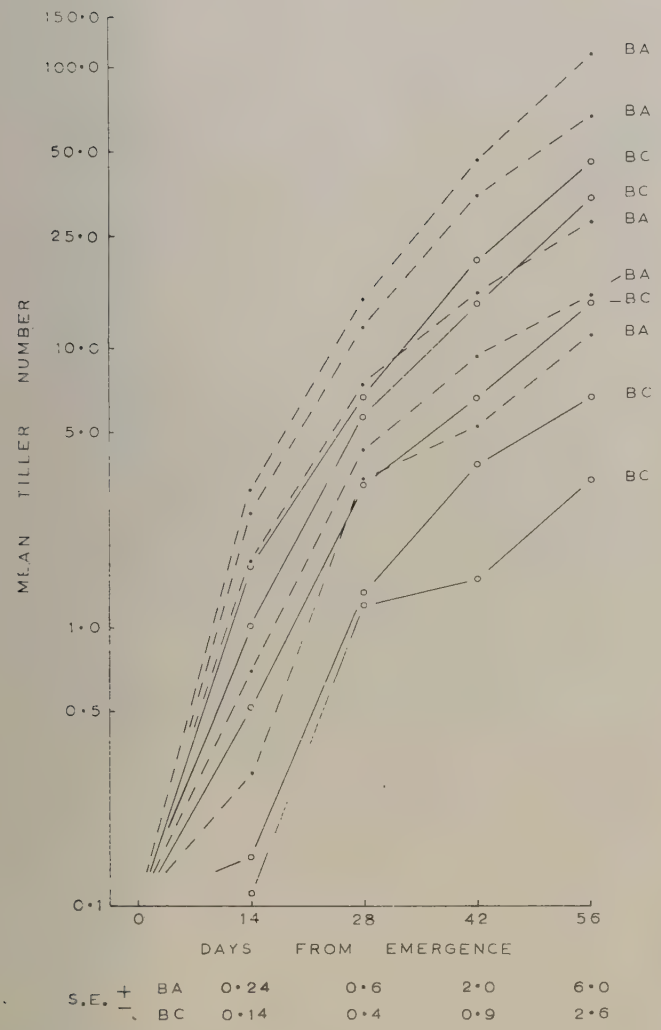

Fig. 2.—Legend: Perennial ryegrass, BA; Cocksfoot, BC. Numbers of tillers produced by single perennial ryegrass and cocksfoot plants in response to nitrogen levels, at four harvest dates.

Leaf Number

Leaf numbers for the four harvest dates are shown graphically in Fig. 3 (log. scale). Average S.E.s are also shown.

The conclusions to be drawn are the same as those given above for tiller number.

TABLE 2. Leaf Areas (sq. cm) of Single Perennial Ryegrass and Cocksfoot Plants at the Four Harvest Dates

Treatment (Equivalents of Applied Nitrogen in lb N per acre)	Harvest 1: 9/12/58		Harvest 2: 23/12/58		Harvest 3: 6/1/59		Harvest 4: 20/1/59	
	Perennial Ryegrass	Cocksfoot	Perennial Ryegrass	Cocksfoot	Perennial Ryegrass	Cocksfoot	Perennial Ryegrass	Cocksfoot
Nil	0.9	0.4	2.6	1.1	7	2	10	4
6	0.8	0.4	6.3	2.9	16	4	23	21
20	1.3	0.6	13.8	6.3	31	25	79	69
60	2.1	1.1	25.2	16.2	101	66	240	228
120	2.9	1.1	53.6	19.0	218	118	571	359

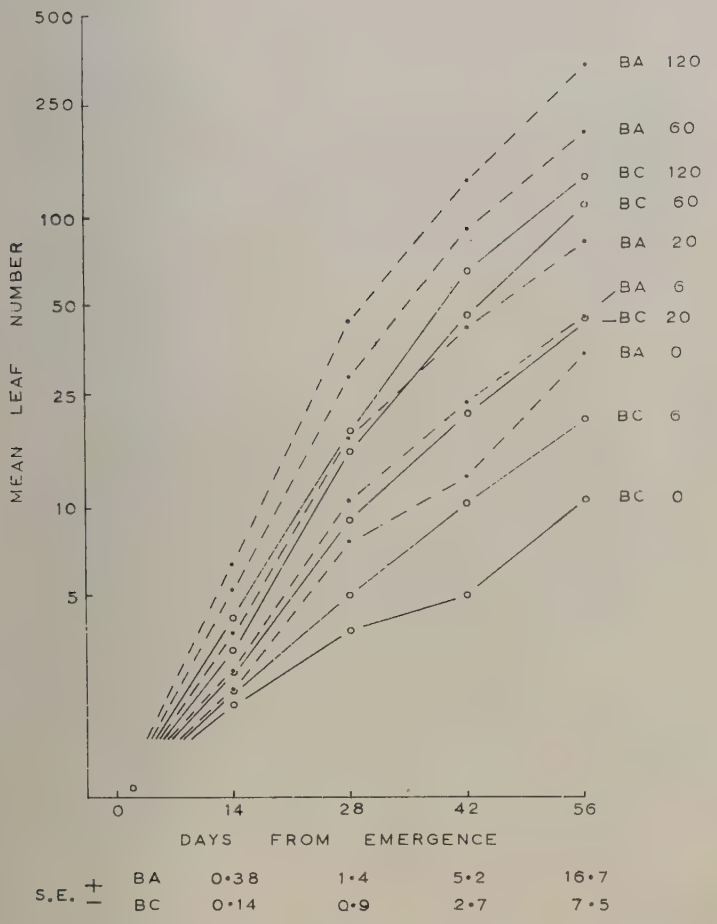

Fig. 3.—Legend: Perennial ryegrass, BA; Cocksfoot, BC. Numbers of leaves produced by single perennial ryegrass and cocksfoot plants in response to nitrogen levels, at four harvest dates.

Leaf Area

Mean leaf areas per plant are presented in Table 2.

The following points are of interest:

(a) The increase of leaf area in perennial ryegrass in response to increasing levels of nitrogen appeared to be twice that of cocksfoot for the first three harvest dates, except at 20 lb N/ac in the third harvest when the increases were similar in the two species.

(b) At the fourth harvest, the differences in leaf area between the species had narrowed appreciably except at the highest nitrogen level, where the difference continued to favour perennial ryegrass.

Relative Growth Rate

Relative growth rates, together with their standard errors, are shown graphically in Fig. 4.

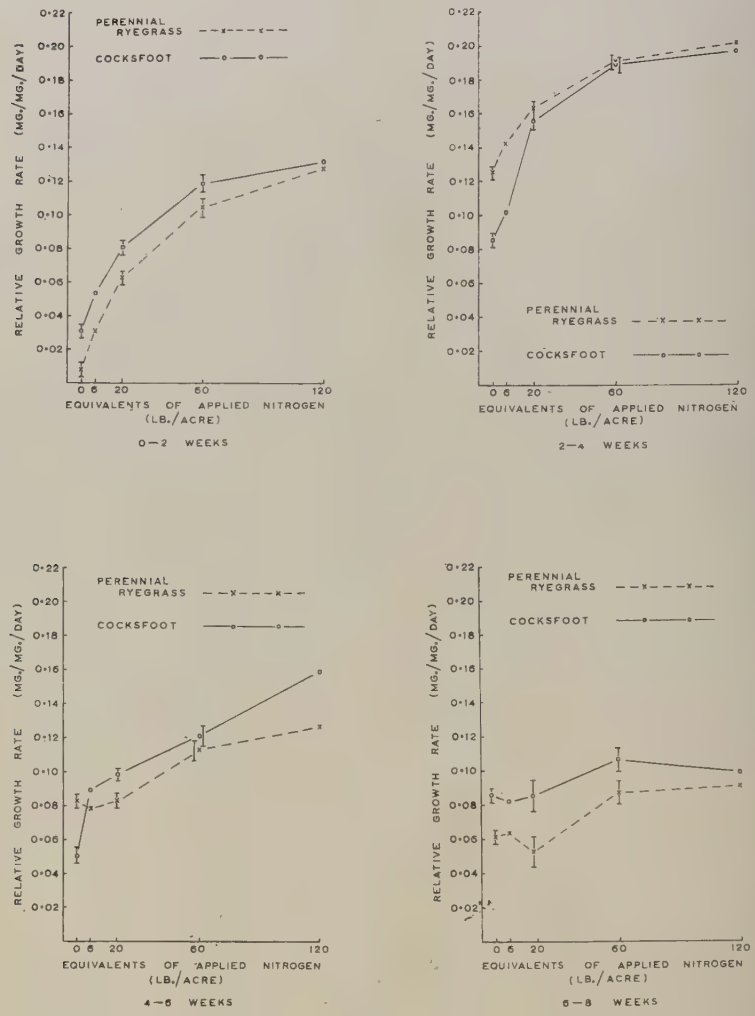

Fig. 4.—Relative growth rates during the four intervals between harvests (mg/mg/day) of perennial ryegrass and cocksfoot as influenced by nitrogen levels.

The following are significant at the 5% level.

(a) At the first harvest, in all except the highest level of nitrogen, cocksfoot was shown to have higher relative growth rates than perennial ryegrass. At the highest level of nitrogen, there was no real difference between their relative growth rates. It should be noted that dry weights of germinated seeds were used as base lines for calculation of these relative growth rates. These seed weights may not represent plant embryos adequately in either species, and may even introduce a bias into species comparisons. Conclusions for this harvest should therefore be treated with caution.

(b) At the second harvest, at the lower nitrogen levels, perennial ryegrass showed higher relative growth rates than those of cocksfoot. At the two higher nitrogen levels there were no real differences.

(c) At the third harvest, at all nitrogen levels except the control, cocksfoot was shown to have higher relative growth rates than those of perennial ryegrass.

(d) At the fourth harvest, cocksfoot had higher relative growth rates than those of perennial ryegrass at the lower levels of nitrogen, but at he highest level of nitrogen the difference did not reach significance.

Discussion

This experiment was carried out during early summer, a time of the year when prevailing temperatures favour the growth of cocksfoot (Mitchell 1956), and the amount of incident light energy available for growth is at a maximum. Moreover, because of the watering procedure used during the experiment, soil moisture levels were at all times high, and adequate for maximum growth. Under these climatic conditions the relative performance of the seedlings and early growth of cocksfoot compared with perennial ryegrass would be as high as could be approached under field conditions. However, even under the experimental conditions, perennial ryegrass showed greater seedling aggressiveness than cocksfoot when judged by plant size and plant yield at all nitrogen levels studied.

Table 1 shows that, at all stages of growth and all nitrogen levels, perennial ryegrass produced more than twice as much weight of shoot tissue per plant as cocksfoot, the difference in favour of perennial ryegrass increasing progressively with nitrogen level. There was one exception to this where ryegrass yielded only one and a half times as much as cocksfoot. Similarly, rate of tiller production and increases in leaf number in perennial ryegrass showed the same trend with respect to nitrogen level. Also increases in leaf area in perennial ryegrass were twice those of cocksfoot for the first three harvests, although, at the fourth harvest, the differences in leaf area between the species narrowed appreciably, except at the highest nitrogen level, where the differences continued in favour of perennial ryegrass.

It is considered that the significant difference in rapidity of tiller and leaf production between the two species is a genetic difference and is due, as suggested by Sharman (1942, 1947) to differences between the potential numbers of leaf and tiller primordia capable of being produced in these two species. Also the contrast in leaf area development between treatments at the same stage of growth was induced, it may be reasonably assumed, by the large differences in the protein nutritional status of the plants. This is in accord with Wardlaw's view (1952) that the dimensions of the leaf are strongly influenced by the nutritional status and hence activity (Mitchell and Soper 1958) and size (Wardlaw 1952) of the sub-apical meristematic tissue.

When the above results are considered in relation to field practice, they show that perennial ryegrass is a more aggressive species in the seedling and early vegetative stage of growth than cocksfoot, even in soils of very low fertility and under conditions where climatic factors favour maximum growth of cocksfoot. At high nitrogen levels, comparable with soils of high fertility, and at all other seasons of the year when conditions are less favourable for cocksfoot, the aggressiveness of perennial ryegrass would be more pronounced.

From the results it would appear that if cocksfoot is to be successfully established, with perennial ryegrass, then low seeding rates, with cocksfoot as the major grass component in the seeds mixture should be employed regardless of soil nitrogen levels.

If plants of equal size are compared, the competitive advantage changed in favour of the cocksfoot after the fourth week of growth at the lower nitrogen levels, owing to the fact that cocksfoot after this time had a higher relative growth rate than perennial ryegrass (Fig. 4).

From the above result, it would appear that a successful method for achieving a balanced ryegrass/cocksfoot/clover pasture on soil of high or medium fertility, would be first to lower the soil fertility by cropping. The mixed grass/clover pasture could then be established by oversowing the ryegrass at a slightly later stage into an already developing cocksfoot/clover sward, so that plants of approximately equal size are obtained when the first grazing is desired.

The sequence of oversowing the ryegrass into a cocksfoot/clover sward would also appear to be of added importance in establishing a new pasture on a soil low in available nitrogen, as certain grass species by virtue of their ready germination and early rapid growth in soils low in available nitrogen are able to compete more vigorously for nitrogen and at an early stage exhaust the limited soil nitrogen supply thereby suppressing the growth of associated species (Sears 1953; Donald 1958). Table 1 shows that cocksfoot would be less competitive toward clovers for nitrogen than ryegrass, at the stage when legumes are dependent upon soil nitrogen for growth before they become independent through symbiotic nitrogen fixation (Wilson 1940).

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ERRATUM. The prices given in the February issue were incorrect, and should be as above.

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